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No. 5, 1949

# CRYSTAL GROWTH

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A GENERAL DISCUSSION  
ON  
**CRYSTAL GROWTH**

12th - 14th April, 1949

A GENERAL DISCUSSION on Crystal Growth was held in the Department of Physics, Bristol University (by kind permission of the Vice-Chancellor) on the 12th, 13th and 14th April, 1949. The President, Prof. Sir John Lennard-Jones, K.B.E., F.R.S., was in the Chair and over 300 members and visitors were present.

Among the distinguished overseas members and guests welcomed by the President were the following :—

Prof. R. Becker (Göttingen), Dr. G. Berkhoff (Geleen, Netherlands), Dr. H. de Bruijn (Geleen, Netherlands), Prof. C. Correns (Göttingen), Dr. P. H. Egli (Washington, D.C.), Dr. P. Franzen (Delft), Dr. W. Gaade (Amsterdam), Mr. I. J. Haven (Eindhoven), Prof. R. Hocart (Strasbourg), Ir. Th. J. J. Hoek (Geleen, Netherlands), Dr. A. N. Holden (Murray Hill, N.J.), Prof. A. Juliard (Brussels), Dr. D. W. van Krevelen (Geleen, Netherlands), Dr. W. C. McCrone (Chicago), Ir. W. May (Delft), Mr. W. M. Mazee (Overleen, Netherlands), Mlle. M. Michel-Lévy (Paris), Dr. S. O. Morgan (Murray Hill, N.J.), Dr. M. H. R. Plusjé (Geleen, Netherlands), Dr. and Mrs. A. H. Spong (Cape Town), Dr. E. W. R. Steacie (Ottawa), Prof. D. C. Stockbarger (Cambridge, Mass.),<sup>\*</sup> Prof. I. N. Stranski (Berlin), Dr. C. E. Sunderlin (U.S. Navy, London), Ir. E. Sweep (Amsterdam), Miss M. G. Ter Horst (Leeuwarden, Netherlands), Mr. R. S. Titchen (Paris), Mr. H. P. J. Wijn (Eindhoven), Dr. J. Willems (Krefeld) and Dr. S. Zerfoss (Washington, D.C.).

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# GENERAL INTRODUCTION ON CRYSTAL GROWTH

BY W. E. GARNER

*Received 31st January, 1949*

In the earlier investigations the two aspects of the growth of crystals, the initiation of crystallization and the rate of growth, were developed independently. It is now realized that each plane of atoms or molecules added to the crystal may involve a fresh initiation of crystallization, and that the rate of crystallization is dependent on the rate of nucleation on the crystal surface. This means that in the fundamental treatment of crystal growth, the two sections are inseparable, and this has been recognized in the grouping of papers for this Discussion.

In this introduction, which is mainly historical, the gradual evolution of the present outlook is indicated. Interest in this field has been accentuated by important applications in industry and a brief survey of these applications is included.

**Initiation of Crystallization.** Throughout the nineteenth century there was much interest in the crystallization of supersaturated solutions, for example, of solutions of Glauber's salt, magnesium sulphate, vitriols, etc. Boisbaudron found that spontaneous crystallization took place only in strongly supersaturated solutions and de Coppet, by cooling solutions, determined the limits of solubility at which spontaneous crystallization begins. Ostwald developed the idea of a metastable zone on the solubility diagram showing the limits within which no crystal nuclei could form spontaneously. This theory proved to be of considerable practical importance at the time in explaining some of the phenomena of precipitation and of Liesegang rings.

Much attention was paid to the limiting size of particle needed to start crystallization in the metastable zone, and rough estimates gave a minimum size of  $10^{-9}$ – $10^{-12}$  g. The thermodynamic criteria developed by Willard Gibbs in 1878 which were applicable to this problem were not very early appreciated, with the result that for a long period the approach to the subject was empirical in character.

Tammann's work on the initiation of crystallization in undercooled organic liquids and inorganic glasses was of the greatest significance and settled many doubtful points. By making counts of nuclei under controlled conditions, he showed that the formation of nuclei obeyed the laws of probability and that the maximum probability occurred at temperatures  $40^{\circ}$ – $120^{\circ}$  below the melting point, where the liquids begin to lose their mobility and show marked changes in viscosity. There was a zone of about  $20^{\circ}$ , below the melting point, where nuclei formation was very slow, which corresponded to the metastable zone found with supersaturated solutions. Tammann showed that nuclei could be formed in this zone if the observer would wait long enough for them. He thought, however, that there might be a metastable region a few tenths of a degree below the melting point, due to an increase in solubility resulting from a decrease of particle size. He also found that the rate of nuclei formation became very slow in the glassy state of undercooled liquids, where the viscosity was very high.

Tammann considered that since the formation of a nucleus was a very rare event, a large number of molecules must meet under limiting conditions; o



velocities, orientation and direction of movement, before a nucleus can be formed. The process was so complicated that any simple relations between the probabilities and the stabilities of the forms produced were not to be expected. He concluded that Ostwald's Law of Stages was not universally applicable.

Willard Gibbs showed that a spherical particle of phase II,  $p = p''$ , was in equilibrium with a continuous phase I,  $p = p'$ , when  $r = \frac{2\sigma}{p'' - p'}$ . The equilibrium is, however, unstable, for if  $r$  is slightly reduced, the particle will decrease in size and finally disappear, and if it be slightly extended it will grow until phase I completely disappears. The work done in the creation of a particle of phase II in phase I is always positive up to the value of  $r = \frac{2\sigma}{p'' - p'}$ , so that phase I is stable with respect to nuclei formation so long as  $r$  is of such magnitude for the surface tension equation to apply. It will break down as  $r$  approaches molecular dimensions and  $p'' \gg p'$ . It would be expected, therefore, that for an undercooled liquid there would be a metastable region for phase I, where spontaneous nuclear formation could not occur, and a metastable limit below which the system became labile owing to  $r$  approaching molecular dimensions.

Haber employed the Thomson equation,

$$\frac{T_s - T_r}{T_s} = \frac{2\sigma M}{rQ_s\rho}$$

in a theoretical examination of the crystallization of supercooled liquids.  $T_s$  is the melting point,  $T_r$  the melting point of a nucleus of radius  $r$ ,  $\sigma$  the interfacial energy,  $Q_s$  the heat of crystallization,  $\rho$  the density of the solid phase, and  $M$  the molecular weight. He postulated a *Spurenschmelzpunkt* as the melting point of the smallest ordered aggregate, which determined the temperature of the metastable limit.

These considerations of Gibbs and Haber will, however, be modified if there be taken into account the local fluctuations of energy which occur in any fluid and which have been demonstrated in the phenomena of critical opalescence. These local fluctuations will facilitate the formation of nuclei and render the metastable limit less sharp, although the conception of a metastable zone is still of some practical value.

**Rate of Growth.** Tammann's researches on the crystallization of supercooled liquids show that the rate of crystallization is very slow down to about  $30^\circ$  below the melting point, increasing to a maximum which is often flat, and falling off as the viscosity increases to that of a glass. The maximum for the rate of crystallization lies at higher temperatures than for nucleation. The low values just below the melting point are due to the slow removal of heat of crystallization. Tammann concludes that the rate is at its maximum when the temperature of the melt is

$$T = T_0 - q_0/c_m,$$

where  $T_0$  is the melting point,  $q_0$  the heat of crystallization, and  $c_m$  the mean specific heat.

**Surface Flow.** Studies of the growth of crystals from the gaseous phase indicate that the flow of molecules over the surfaces of the crystals plays an important role in the rate of crystallization. Volmer and Estermann showed that mercury crystals formed from the vapour consist of very thin flat plates, and that the rate of extension of the main faces can only be accounted for if the molecules colliding over the whole surface of the crystal are available for the growth of the very small areas at right-angles to the basic planes. This requires that the surface flow of a molecule during its

lifetime on the surface is of considerable magnitude. The work of Becker and of Taylor and Langmuir on adsorbed caesium on tungsten, and of Bosworth on potassium on tungsten, at temperatures where the evaporation of the adsorbed atoms is low, shows that the atoms undergo activated diffusion along the surface. For caesium the number of sites covered during the lifetime is at least  $10^8$ . Also, Newman has demonstrated that activated diffusion occurs on the surface of heated sodium chloride crystals. The experiments of Volmer and Adikari on the surface flow of benzophenone on glass and of Kowarski on *p*-toluidine over a crystal of the same substance illustrate the same principle.

The extension of this principle to crystallization from supersaturated solutions and from undercooled melts is unavoidable, since in general the work required to move a molecule or ion along the surface is less than that to transfer it to the liquid phase.

**The Repeatable Step.** The energies required to remove ions or molecules of sodium chloride from the surface of a crystal into the gaseous phase have been calculated by Kossel and Stranski for the corner, edge and various surface positions. Homopolar lattices have been dealt with similarly by the same authors and by Becker and Döring. The difference between the energies for the various sites is sufficiently great to have an important bearing on the kinetics of crystal growth.

In building up a plane of atoms on the surface of a crystal, the greatest energy is liberated at the repeatable step of an uncompleted edge of a covered area. The energy evolved on adsorption on such sites is approximately the same as that resulting from embedding the atom half-way in the crystal. The process of crystallization on surfaces large compared with the atomic diameter consists mainly in the repetition of the 'repeatable step.' The adsorption of atoms singly on the plane surface is much less strong than at the repeatable step. Over part of the range of temperatures for which atoms are firmly held at the repeatable step, those on the main surface are readily desorbed. The surface molecules, however, travel by surface flow considerable distances before they evaporate, and therefore it is to be expected that in favourable circumstances the whole surface of the crystal will act as a collecting ground for the repeatable step.

**Two-dimensional Nuclei.** The rate of evaporation is greatest if the adsorbed molecules are held singly on the surface and least when held at a repeatable step on a two-dimensional nucleus, the size of which is above a critical value. In the building-up of new crystal planes, the average time taken to complete a two-dimensional nucleus of this critical size may be considerably greater than that required to complete the plane of molecules by a succession of repeatable steps. Volmer, for iodine crystals growing from vapour, concludes that the formation of the two-dimensional nucleus is such a rare event that the probability of its occurrence determines the velocity of crystallization.

Crystals grow the more regularly the lower the supersaturation. At high supersaturations polymolecular sheets are built up, giving a series of steps on the faces of crystals which can be detected by interference colours (Marcellin, Perrin, Kowarski). These phenomena are of frequent occurrence and are of special interest. Stranski, studying the growth of polished spherical surfaces, shows that the planes with high indices of even simple lattices give uneven surfaces during growth, built up of steps of various heights. It should, however, be borne in mind that some of these phenomena may be due to the discontinuities caused by polishing. It is clear, however, that the mechanism of crystal growth, with complex molecules from strongly supersaturated solutions, can become an involved problem. Phenomena make

their appearance which have not been unambiguously elucidated. It is possible that some of these may be due to Smekal, Zwicky or other types of discontinuity, as suggested by Frank. However, under the simplest conditions, with low supersaturation, the conception of the formation of two-dimensional nuclei aided by surface flow may prove to be adequate for the calculation of rates of growth.

**Crystal-Crystal Interface.** The nuclei formation in solid phases obeys similar temperature relationships to supercooled melts, giving maxima at temperatures considerably below the melting point. Volume changes on crystallization, producing cracks, are, however, an added complication. Nuclei formation in processes which are accompanied by gas evolution are one step more complicated, but the phenomena obey the same general rules. In a number of cases in which gas evolution occurs, the activation energy is approximately the same as the thermodynamic heat for the process, which implies a close fit between the lattices of the two phases and a very close coupling between the disappearance of the old and the building-up of the new lattice. This may well be the case, in favourable circumstances, for the growth of one crystal phase out of another.

**Practical Applications.** The need for large crystals free from flaws for spectroscopy, piezoelectric measurements and the various purposes of the electrical industry cannot be met from the diminishing natural resources, nor do these give a sufficient variety. This has led to researches on the methods of accurate control of crystallization from the vapour phase, the melt, from supersaturated solutions and by hydrothermal processes at high pressures simulating those in nature. In the natural processes whereby crystals are formed in the earth's crust, an infinitude of time is available for the manufacture, but on the industrial scale the time available makes it necessary to work at higher supersaturations, where irregularities are the more likely to occur in the crystallization processes.

The control of crystal shape and size by the addition of surface active substances is a requirement in many industries. In the explosives industry particles with as nearly spherical shape as practicable are advantageous from the point of view of flow properties, bulk density, pelleting properties, etc. It is also possible in cases where two solid modifications are produced to prevent the formation of the unstable modification by the use of suitable additaments. The control of particle size distribution is also important in the manufacture of materials used as the basis of products with good plasticity. The tendency of hygroscopic substances to cake can often be reduced by paying attention to crystal shape, choosing that shape which gives a minimum of contacts between the grains.

The surface agents may operate by adsorption on one set of faces, either reducing or preventing growth, as is found by the use of certain dyestuffs. These agents may operate by retarding all growth except in one direction, thereby giving spherulitic growths. The detailed mechanism by which they act is not yet elucidated, although it can readily be seen from current ideas on crystal growth that the effects of adsorption at the repeatable step would have important consequences.

There are many processes in which crystallization is the final stage, giving the product its essential properties. Such are the manufacture of cements, bricks, ceramics, etc. Although in these cases the crystallization process is often accompanied by chemical change, the mechanism involves the nucleation by crystals and the growth of crystals such as occurs for the simpler processes, and their study will benefit by the development of the fundamental theory of crystal growth.

*The University, Bristol.*

# I. THEORY OF CRYSTAL GROWTH

## Introductory Paper

By N. F. MOTT

*Received 7th March, 1949*

The theory of crystal growth can, it seems to me, conveniently be divided into three parts. These are :

(a) The theory of the rate of growth of a surface in contact with a vapour or solution with a given degree of supersaturation. Or, in the case of a crystal growing from the melt, the theory of the rate of growth for a given degree of supercooling. This will include a discussion of the rates of growth of different crystal faces, and the effect on growth rates of impurities which may be adsorbed on the surface, and of imperfections in the crystals themselves. The solution of the problems under this heading depends, of course, on a knowledge of interatomic forces.

(b) The use of results obtained under the heading (a) to determine crystal forms in as far as they depend in the case of growth from solution, or diffusion of the ions or atoms to be deposited, or in the case of growth from the melt on conduction through the material of the heat liberated. Much of the theory of dendrite formation is included in this category. It forms a part of classical rather than atomic physics, depending as it does on the equations of diffusion and heat flow.

(c) Discussions of the crystal form of the deposit. This will include such problems as the formation during growth of screw or edge dislocations in the crystal ; a solution of these problems is very important for the theory of mechanical strength. Then there is the question of the possible pseudo-morphic forms of crystalline films grown on a substrate of different composition ; a contribution to this subject is made by van der Merwe in a paper to be presented to this conference. And, finally, there is the question of the state of strain and possible cracking of the surface layer treated by Molière, Rathje and Stranski.

(a) **Atomic Theory of Growth.** The elements of a theory of crystal growth have been laid down by Volmer, Stranski, Becker and Döring, and new contributions made by Frank, Burton and Cabrera (for references, see the contribution of F. C. Frank to this Discussion). This theory applies explicitly to growth from the vapour ; but can probably be applied in principle to growth from solution. The problem of growth from the melt remains an open question.

The elements of the theory of growth are as follows : consider a flat crystalline surface of low indices (say, (100) for a simple cubic or (111) for a close-packed structure) in contact with a vapour. Suppose this surface is partly covered by another layer. Then if the pressure of the vapour is raised by a small amount  $\Delta p$  above the equilibrium vapour pressure, theory indicates that the layer will grow, with a speed proportional to  $\Delta p$ , until it covers the surface. But in order to start a new layer, a two-dimensional nucleus must be formed, and, like other nucleation phenomena, the rate of nucleation varies with  $\Delta p$  as  $e^{-A/\Delta p}$ , where  $A$  is a constant at given temperature. It follows that when  $\Delta p$  is below some critical value the rate is negligibly small.

It seems likely that the growth rate depends in general on the rate of nucleation, at any rate for surfaces of low indices ; for surfaces of high indices, having a step-like formation anyhow, nucleation is much easier.

But such surfaces of high indices will, of course, by growing quickly tend to disappear, leaving a crystal surrounded by planes of low index only.

It should be emphasized that a flat surface in contact with vapour will have a number of atoms adsorbed on it. Two-dimensional nucleation can occur whether or not these are mobile over the surface; it is not at present quite certain whether their mobility affects the rate of nucleation.

Among the papers presented to this Discussion, Becker gives a valuable account of the relation of his theory to Mayer's theory of condensation. Burton and Cabrera, in a paper to be published elsewhere, have made some refinements to the present theory by calculating the shape of the two-dimensional nucleus when it has reached the size beyond which it will normally spread. This puts the theory on a firmer footing, and does not alter the numerical values very much. Frank points out that the theory suggests a growth rate which is negligibly small unless the supersaturation of the vapour is of the order 1.5, and that this is contrary to experiment, in particular to the results of Volmer and Schultze on the growth of iodine crystals; the degree of supersaturation required is of the order 1.01. He suggests that the presence of dislocations is essential for growth at these concentrations, and that the growth rate depends essentially on the density of dislocations in the material.

Theory has at present made little contribution to our knowledge of habit modification. It does, however, follow that, if dislocations are essential for crystal growth, very small concentrations of impurity, which could be adsorbed preferentially at the "ledge" where the dislocation meets the surface, could profoundly affect growth rates and thus lead to habit modification.

(b) **Phenomena Depending on Heat Flow and Diffusion.** It is believed that dendrite formation in the solidification of liquid metals is due to the fact that a thin needle, growing into a supercooled solution, will need to get rid of less heat by conduction than a thicker one and so will grow faster. In the same way, in the formation of crystals from solution, a thin needle will grow more quickly than a thick one into supersaturated solution. Probably the clue to the step formation observed by Bunn will be found along these lines.

(c) **Physical State of the Crystal as a Consequence of the Mechanism of Growth.** Frank, in his paper, gives some reasons for believing that, at finite growth rates, dislocations will be formed in the crystal. They are in no sense present in thermodynamic equilibrium and ideally a long enough anneal would get rid of them; but, in practice, there appear always to remain a certain number.

Stranski and his colleagues reopen the very interesting question of the state of strain of the surface layer. The origin of the "Griffith cracks," responsible for the low stress for fracture of brittle materials, has never been explained, and it is possible that this work will provide a clue.

In a later section of the Discussion, van der Merwe discusses the crystal structure of thin films deposited on a substrate of differing crystal structure. He shows that the question, whether or not the deposit has a pseudomorphic form, depends on whether the first monolayer conforms to the structure of the substrate or not; and that this in turn depends on the degree of misfit.

### **Equilibrium Crystal Forms**

The study of the shape of a crystal in equilibrium with a vapour forms an interesting field rather apart from the theory of crystal growth. Burton and Cabrera have found that the equilibrium form of the two-dimensional crystalline nucleus on a flat substrate is a rounded polygon, if only one

atomic or molecular unit is involved. For ionic forces, on the other hand, it appears that the two-dimensional nucleus may have sharp corners. In the case of three dimensions Stranski has shown that the corners of a crystal are rounded off through the presence of a *finite* number of planes of higher index, and so are not truly rounded.

The microstructure of the surface in equilibrium with vapour or solution is also of interest. As already stated, a flat surface will always contain some adsorbed atoms, and there will always be some vacant lattice points. Burton and Cabrera have made an investigation of the concentration of "Frenkel terraces" on a surface in equilibrium. For faces of low index, there will be practically none for a perfect crystal; any which exist depend on the presence of dislocations. A crystal temperature exists, however, at which they form, but this will in general be above the melting point.

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## FORMS OF EQUILIBRIUM OF CRYSTALS

BY I. N. STRANSKI

*Received 17th February, 1949*

A knowledge of the forms of equilibrium of crystals is important for an understanding of the processes on crystal surfaces, independent of whether the crystal is immediately concerned in these, or merely functions catalytically. Furthermore, a comparison between theoretically and experimentally deduced forms of equilibrium makes it possible to check the assumptions used in the former, and supplies valuable clues to alteration in structure and changes within the individual lattice surfaces.

The following observation should first be made. The theoretical treatment falls into two parts. First the underlying ideas must be developed, then the mathematical work can be started. This is directed by the knowledge at the time of the force functions, and must of necessity bring new problems in its train. In the following I will confine myself to the part dealing with the underlying ideas.

The treatment of the forms of equilibrium of crystals has been developed on the basis of two fundamentally different ideas. The older one, historically, made use of an analogy to liquid surfaces. The surface tension here was replaced by the idea of the specific surface energy  $\sigma$ .

The values of  $\sigma$  for crystals are dependent upon direction, so that in general the form of equilibrium is a polyhedron which must satisfy Gibbs' condition:

$$\sum \sigma_i \cdot F_i = \text{minimum, at constant volume.}$$

If one ignores the edges and corners, it is known that here, also, one arrives at the same relation as for vapour pressure, which is completely analogous to Thomson's equation and has the following form<sup>1</sup>:

$$\frac{kT}{2v_0} \ln \frac{p_r}{p_\infty} = \frac{\sigma_1}{r_1} = \dots = \frac{\sigma_i}{r_i} = \dots \quad (1)$$

<sup>1</sup> The following recent papers on the Thomson-Gibbs relation are mentioned:  
Volmer, *Kinetik der Phasenbildung* (Dresden and Leipzig, 1939), p. 87 *et seq.*  
v. Laue, *Z. Krist.*, 1943, **105**, 124.  
Stranski, *Z. Krist.*, 1943, **105**, 91.  
Honigmann, Molière and Stranski, *Ann. Physik*, 1947, **1**, 181.

$v_0$  represents the volume of a crystal unit and  $r_i$  the centre distance,  $\sigma_i$  the specific surface energy of the  $i$ -th face.  $p_f$  and  $p_\infty$  are the sublimation pressures of the finite- and infinite-sized crystals respectively. Wulff's method for the construction of equilibrium forms of crystals follows directly from eqn. (1).

An exact relation, capable of general application, cannot be derived in this way. For if we wish to take into account the fact that the crystal also possesses edges and corners, and that the specific surface energy and the specific energy of the edges and corners which must further be introduced,

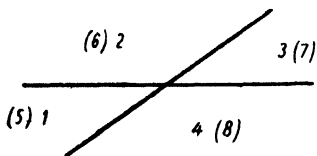


FIG. 1.—Division of a crystal by three planes. Two of these are shown as lines where they cut the plane of the diagram, the third lies in the diagram plane itself. The figures in brackets denote the sections below the plane of the diagram, those without brackets the sections above it.

possibly depend on the size of the crystal as well, a relation can only be derived at first for simplified models. This is to be shown in the following for the case where the form of equilibrium of the crystal is represented by a simple crystalline form, i.e., it is surrounded by only one kind of face. For this purpose let us refer to the definition of the specific surface energy, and give the definition of the specific edge and corner energies in reference to Born and Stern.<sup>2</sup>

The specific edge energy  $\chi$  is defined as the work which must be done in order to separate the crystal sections 1 and 3, 2 and 4 respectively (see Fig. 1), divided by twice the length of the edge, and given a negative sign. Correspondingly, the specific corner energy  $\epsilon$  is half the work required to separate two crystal sections situated diagonally in space, with their corners touching, e.g., 1 from 7, or 3 from 5 (see Fig. 1).

Assuming that these values are independent of the dimensions of the crystal, one obtains in place of eqn. (1) :

$$\frac{kT}{2v_0} \ln \frac{p_f}{p_\infty} = \frac{\sigma}{r} + \frac{\chi}{2r^2} \quad (2)$$

Thus, as a result of the existence of edges, an additional term appears as correction. The corners are without influence.

In order to be able to discuss the dependence of the values  $\sigma$ ,  $\chi$  and  $\epsilon$  upon the size of the crystal at all, the definitions of these values for finite crystals had first to be found. The definitions given by me at that time<sup>3</sup> will be explained for a simple case with the aid of Fig. 2. If the form of equilibrium is represented by a cube,  $\sigma_a$  is equal to the work of separating such a small crystal from a cube face of the infinite crystal, divided by twice the area of one cube face of the small crystal.  $\chi_a$  is correspondingly equal to the work of separating such a cube from the infinite crystal quadrant lying diagonally opposite divided by twice the length of a single edge and

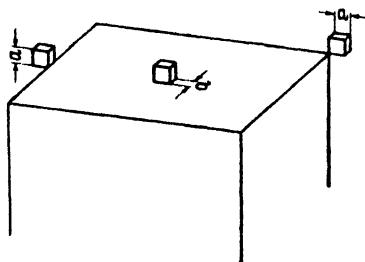


FIG. 2.—To define the values  $\sigma_a$ ,  $\chi_a$  and  $\epsilon_a$  for a finite crystal cube with an edge-length  $a$ .

<sup>2</sup> Born and Stern, *Ber. Berlin Akad.*, 1919, **48**, 91; Stranski, *Z. Krist.*, 1943, **105**, 287.

<sup>3</sup> Stranski, *Ber. Wien. Akad., math.-naturwi. Kl.*, 1936, **11b**, **145**, 840; *Mh. Chem.*, 1936, **69**, 234.

with a negative sign. Lastly the corner energy  $\epsilon_a$  is equal to half the work of separating a small crystal from the infinite crystal octant lying diagonally opposite in space. Thus the total surface energy of a small crystal with edges of length  $a$  is

$$\Phi_a = 6a^2\sigma_a + 12a\kappa_a + 8\epsilon_a. \quad (3)$$

In this case it is possible to obtain the form of equilibrium of a small crystal simply by taking an infinite crystal to pieces, and  $\Phi_a$  can also be defined as the work of separating a small crystal from a crystalline half-crystal position (see Fig. 3). However, it should be mentioned that  $\Phi_a$  is generally given in the following relation :

$$\Phi_a = N_a \cdot \varphi_{1/2} - \sum_{\nu=1}^{N_a} \varphi_{\nu}. \quad (4)$$

$\varphi_{1/2}$  is the work of separating a crystal unit from the half-crystal position (see below). The second term is the work obtained in building up the small crystal from its  $N_a$  individual crystal units.

The example dealt with in the last section is especially simple. The important thing is, that this case already shows that it is not possible to specify the exact sublimation pressure of a small crystal from the forms of equilibrium, with the aid of the values  $\sigma_a$ ,  $\kappa_a$  and  $\epsilon_a$ , now assumed to be variable.

For this purpose, the differentiation of the eqn. (3) is necessary :

$$kT \ln \frac{p_a}{p_{\infty}} = \frac{d\Phi_a}{dN} = 12\sigma_a a \frac{da}{dN} + 6a^2 \frac{d\sigma_a}{dN} + 12\kappa_a \frac{da}{dN} + 12a \frac{d\kappa_a}{dN} + 8 \frac{d\epsilon_a}{dN}. \quad (5)$$

The values  $\Phi_a$ ,  $\sigma_a$ ,  $\kappa_a$  and  $\epsilon_a$  would thus have to occur as continuous functions of the number of crystal units  $N$ . That is not the case, however, for they present themselves as a series of isolated points.

The following possibilities can be discussed. (1) Curves are drawn through these points and differentiated. The result could give the sublimation pressure with sufficient exactitude. (2) The dependence of the values  $\sigma_a$ ,  $\kappa_a$  and  $\epsilon_a$  upon  $N$  can be found to be so small that it can be neglected. Neither possibility, however, can be proved for no standard of comparison exists at present, which gives us the correct pressure values. We will return to these questions below.

The advantages of the method using the values  $\sigma$ ,  $\kappa$  and  $\epsilon$  are not to be denied, for by means of it, all considerations which had been made on liquid systems could be applied in a comparatively simple way, and with little alteration, to crystal systems. Special attention is here drawn to the fact that, on the whole, Volmer's theory on the frequency of nucleus formation<sup>4</sup> also reproduces the conditions correctly for crystal systems. By continuing the nucleus idea, introducing, namely, the idea of a two-dimensional nucleus, the growth of a crystal could be submitted for the first time to a mathematical method. Many different questions could be answered comparatively simply. The interpretation of Ostwald's step-rule may be mentioned as an example.<sup>5</sup>

But the disadvantage of this method must also be enumerated. The values  $\sigma$ ,  $\kappa$  and  $\epsilon$  do not refer at all to elementary stages of growth and reduction, and the relations which are obtained with their aid can only be applied under certain conditions to kinetic considerations on crystals, and remain difficult to visualize. As is known, the application of Thomson-Gibbs'

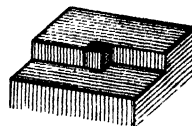


FIG. 3.—Crystalline half-crystal position.  $\Phi_a$  is equal to the work of separation of the crystal cube with edge-length  $a$ , in the half-crystal position, as shown in the diagram.

<sup>4</sup> Volmer and Weber, *Z. physik. Chem.*, 1926, **119**, 277; Volmer, *loc. cit.*

<sup>5</sup> Stranski and Totomanow, *Z. physik. Chem. A*, 1933, **163**, 399.



equation has led to numerous, and often crass, misunderstandings. This method, by simulation of completion, has also prevented many equilibrium questions from being asked and answered at the right time.

The second treatment began to take form as a result of work by Kossel<sup>6</sup> on the one hand, and myself on the other.<sup>7</sup> The work of separating individual crystal units from the crystal surface was estimated, NaCl being taken as the first example, and with the help of this it was possible to draw a picture of the molecular processes connected with growth and solution. The logical starting point for these considerations is the determination of the length of time a crystal unit remains in the so-called half-crystal position.

The crystal unit in the half-crystal position possesses a work of separation which amounts to half of that of a crystal unit in the inside of the crystal. It is thus equal to the negative value of the lattice energy per crystal unit, and determines the vapour pressure of the infinite-sized crystal. Elementary reasons can be given for this conclusion if the position of growth of a repeatable growing crystal face is chosen as model of the half-crystal position. For, in this case, the position as such is retained after any number of separations or addition of crystal units. Thus the crystal would only be in equilibrium with its surroundings, if the probability of a separation of a crystal unit from the half-crystal position is found to be equal to the probability of an addition on this.

With the help of the different works of separation, it has already been possible to draw a series of conclusions which, at that time, were justifiably regarded as completely new-fangled. Only as a consequence of these was it asked whether certain faces in equilibrium can be retained as such, or whether their surface structures would have to undergo alterations of a coarsening nature.

It should be emphasized here that these questions could have been asked earlier, as a result of the determination of the values, or merely the signs, of the specific peripheral energies of the lattice surfaces concerned. That they have not been asked up to this time is to be explained exclusively by the fact that the older theories were difficult to visualize.

Because of its importance the criterion might be given here by reason of which one can decide whether a certain face appears in the equilibrium form of the infinite-sized crystal or can remain as crystal face. If the specific peripheral energy of this lattice face shows the value zero or a negative value, in one direction only, this face cannot appear as a form of equilibrium. Should this condition be fulfilled for one direction only, the face concerned will grow over one-dimensional nuclei and show a typical chain formation. A chain formation alone, on the other hand, is not sufficient argument against the face belonging to the equilibrium form. If this condition is fulfilled for two directions, the one-dimensional nucleus formation is also eliminated. An example of the first case is (011) on the NaCl crystal and of the second, (111) on the same crystal.

Another question could also be answered with the help of the work of separation, namely, with what kind of face must the infinite-sized crystal be surrounded? For it is evident that the only possible form of equilibrium is one in which all corner crystal units are bound at least as firmly as in the half-crystal position. So that by starting with a simple form, and systematically removing all crystal units which are less firmly bound, one could arrive at forms which no longer exhibit such crystal units, and which then mirror the equilibrium form, in that they possess all the faces of same.

In order to arrive at an expression which represents the sublimation

<sup>6</sup> Kossel, *Nach. Ges. Wiss. Göttingen*, 1927, 135; *Leipziger Vorträge*, 1928, 1.

<sup>7</sup> Stranski, *Z. physik. Chem.*, 1928, 136, 259.

pressure of finite crystals, those crystal units will be taken into consideration which, on evaporating, produce a deviating value for the work of separation. In the case of a single crystal face, that is a very simple matter.<sup>8</sup> The mean value  $\bar{\varphi}_a$  appears here in place of  $\bar{\varphi}_\infty = \varphi_{1/2}$  ("work" of separation of the crystal unit in the half-crystal position), where the mean is taken so as to include all crystal units of the uppermost lattice face, and for a process also, in which the crystal units of the lattice face are removed. The logarithm of the relation between this vapour pressure and that of an infinitely extended lattice face is then simply

$$kT \ln \frac{p_a}{p_\infty} = \varphi_{1/2} - \bar{\varphi}_a. \quad (6)$$

This simple result can be explained as follows: for the faces concerned in equilibrium, the probability that the uppermost lattice face is removed by solution must be equal to the probability that, after removal, it is reformed by means of a condensation process. But this stipulation is connected with the fact that the work of formation of a lattice face nucleus (two-dimensional nucleus) by condensation is exactly equal to that by superficial solution of an uppermost lattice face.

Let us imagine a position where a crystal unit is bound in such a way that the work of separation has the exact value ( $\bar{\varphi}_a$ ) required for the position to be occupied by a crystal unit for not more than exactly one-half of a very long observation period. We could then undertake the formation of a lattice face nucleus by condensation, by allowing the crystal units to attach themselves first at this point from the vapour phase, and forming the nucleus by bringing them each time from there on to the face. If the nucleus contains  $m$  crystal units and the whole lattice face  $n$ , the following work is necessary for the production of the nucleus:

$$\sum_1^m (\bar{\varphi}_a - \varphi_i).$$

By superficial solution of an existing lattice face, on the other hand, the work of formation of the nucleus amounts to

$$\sum_{m+1}^n (\varphi_i - \bar{\varphi}_a).$$

By balancing the two work equations, one obtains directly

$$\bar{\varphi}_a = \frac{1}{n} \sum_1^n \varphi_i. \quad (7)$$

The following should also be taken into account. The conditions of equilibrium deduced quite generally apply to both lattice faces and single lattice rows. In the case of the lattice face nuclei, the peripheral rows must be in equilibrium with the surroundings, i.e., the mean work of separation per peripheral row of the lattice face nucleus must show the same value  $\bar{\varphi}_a$  on all sides.

If we now consider a three-dimensional crystal which is in equilibrium with its surroundings, this implies that the same conditions must be fulfilled for each of its faces. From which it further follows that the surroundings are supersaturated as regards all rows on the edges of the crystal (for the uppermost lattice face of an equilibrium form is greater than the corresponding lattice nucleus); in the same way, the surroundings are also supersaturated as regards each single point on the surface of the crystal, and

<sup>8</sup> Stranski and Kaischew, *Z. physik. Chem. B*, 1934, **26**, 100, 114, 312; *Physik. Z.*, 1935, **36**, 393.

therefore also as regards the corner crystal units. This conclusion is instructive. But it is also fundamental for the consideration of the equilibrium of a crystal. It leads directly to an easy method of construction of the form of equilibrium of crystals. In order to obtain the form which corresponds to a certain pressure, in the vapour phase, one proceeds as follows: the value of  $\bar{\varphi}_a$  corresponding to the pressure  $p$ , is calculated. Then starting from any simple form of the crystal, all crystal units which show a work of separation smaller than  $\bar{\varphi}_a$  are eliminated, one after the other, from its surface. Lastly, the areas of all faces are varied until each single mean work of separation reaches the value  $\bar{\varphi}_a$ .

Another conclusion from the thermodynamic deduction of the sublimation pressure of a small crystal is made especially clear. That is the conclusion which can be drawn directly from eqn. (2): the vapour pressure is simply a function of the relation between  $\sigma$  and the centre distance of any face. Provided the latter remains the same this quotient must remain unchanged independent of whether the face concerned occurs in a simple form or in a combination. When drawn from the thermodynamic deduction, this conclusion is not clear, as the deduction includes only the form of equilibrium itself, and is tied to the assumption that for small evaporation and growth processes the form remains similar. The following explanation, based on the mean work of separation, can be given for this conclusion. To this end, let us begin with a simple form and study a definite face. This form is now allowed to develop into a combination, the centre distance of the face under consideration remaining unchanged. The area of the face decreases but the deviations of the individual works of separation also decrease to the same degree, for the rows on the edges of the combination border upon more lattice neighbours than the rows on the edges of the simple form.

Lastly, eqn. (6) provides the possibility of deciding the question which cropped up on a previous page. It supplies the vapour pressure in a manner which is quite independent of that in eqn. (1) or (5). It is also possible, in this manner, to carry out the calculation for a definite example, namely, for a *simplified* NaCl crystal.<sup>1</sup> It showed, though only for this case, that the second possibility is realized, namely, that it is not necessary to include the dependence of the specific energy values  $\sigma$ ,  $\kappa$  and  $\epsilon$  on the number of crystal units, in the calculation, for all crystal sizes which actually come into question.

It is comparatively easy to obtain the form of equilibrium theoretically for typical ionic crystals, if simplifying assumptions are made. In all cases dealt with up to now, it has been found to be a simple form. It is

- a cube for NaCl<sup>2, 6, 7</sup>,
- a rhombic dodecahedron for CsCl<sup>9</sup>,
- an octahedron for CaF<sub>2</sub><sup>10</sup>,
- a rhombohedron for CaCO<sub>3</sub> or NaNO<sub>3</sub>.

It is also independent of the size of the small crystal. Thus, form of equilibrium and form of growth are here identical (for low supersaturations).

The conditions in the case of non-polar crystals are different. In this case the greater the range of the forces between the crystal units, and the nearer  $\bar{\varphi}_a$  approaches  $\bar{\varphi}_\infty$ , the greater the number of faces appearing in the form of equilibrium.

Table I gives a list of (infinitely great) forms of equilibrium for a few simple lattices as functions of the said range and calculated under the assumption that the work of separating one crystal unit from another is always

<sup>9</sup> Kleber, *Zbl. Miner., Geol., Paläont. A*, 1938, 363.

<sup>10</sup> Bradistilov and Stranski, *Z. Krist.*, 1940, 103, 1.





FIG. 4.— Cd mono-crystal, formed in the fused liquid and allowed to grow further in the vapour. The small circular face at the bottom left-hand corner is  $\{11\bar{2}0\}$ , the irregular coarsened face above it  $\{11\bar{2}1\}$ . (Eisenloeffel.)<sup>15</sup>

positive and only dependent upon the distance between them.<sup>11</sup> The majority of crystals with simple lattices would seem to represent transition stages between the polar and non-polar type. The metals constitute a special class. It is worthy of note that the experimental data for metals<sup>12 13</sup> also agree well, on the whole, with the results in the Table, inasmuch as they give the correct order of the faces. It was possible to make a more accurate experimental investigation especially in the case of Zn<sup>14</sup> and recently also for Cd<sup>14 15</sup>, and these results were confirmed. In both cases the experiments on growth, carried out accurately on spherical rudimentary forms consisting of one crystal, which grow from supersaturated vapour without

TABLE I  
FORMS OF EQUILIBRIUM

The range of the forces between the lattice crystal units embraces		Adjacent crystals units only	Next crystal unit but one, also	Next crystal unit but two, also	Investigated on
Lattice type	Simple cube ..	001	001, 011, 111	001, 011, 111 112	
	Body-centred, cubic	011	011, 001	011, 001, 112 111	W, urotropine
	Face-centred cubic	111, 001	111, 001, 011	111, 001, 011 113, 012, 135	Al, Ag, Pt
	Diamond lattice..	111, 001	111, 001, 011	111, 001, 011 113	diamond
	Hexagonal closely packed spheres	0001, 1011 1010	0001, 1011 1010, 1120 1012	0001, 1011 1010, 1120 1012	Be, Mg, Zn, Cd

The most far-reaching effect was found in every case to embrace the next crystal unit but two. The underlined examples have been investigated more thoroughly.

any signs of coarsening, gave the faces: (0001), (1011), (1010); (1120), (1012). It is also very significant that the faces (1120) and (1012), which are to be traced back to the influence of nearest neighbours but one, exhibit a considerably smaller area in the case of Cd, than in the case of Zn (see Fig. 4). This is probably connected with the greater screening capacity of the Cd atoms in the crystal lattice.

It is also very noteworthy that W<sup>16 13</sup> and urotropine<sup>17 18</sup> which both have the same lattice (cubic body-centred) but belong otherwise to quite different valency types, exhibit exactly the same equilibrium form faces: {011}; {001}; {112}. In both cases, of the two faces which are to be traced back to the effect of nearest neighbours but two ({112} and {111}), only {112} appears. Concerning further properties of the urotropine crystal,

<sup>11</sup> Stranski, *Z. physik. Chem. B*, 1931, **11**, 342; *Ber.*, 1939, **72**, 141; Stranski and Kaischew, *Z. Krist.*, 1931, **78**, 373; *Ann. Physik*, 1935, **23**, 330.

<sup>12</sup> Straumanis, *Z. physik. Chem. B*, 1931, **13**, 317; 1932, **19**, 64; 1934, **26**, 246.

<sup>13</sup> Stranski and Suhrmann, *Ann. Physik*, 1947, **1**, 153.

<sup>14</sup> Kaischew, Keremidtschiew and Stranski, *Z. Metallkunde*, 1942, **34**, 201.

<sup>15</sup> Eisenloeffel, *Dissertation* (Techn. Universität Berlin-Charlottenburg, 1948).

<sup>16</sup> Müller, *Z. Physik*, 1937, **106**, 541; 1938, **108**, 668; 1943, **120**, 270.

<sup>17</sup> Kaischew, *Jahrb. Univ. Sofia, phys. math. Fak.*, XLIII, 1946/47, **2**, 99.

<sup>18</sup> Stranski and Honigmann, *Naturwiss.*, 1948, **35**, 156.

whose lattice can be considered approximately as homopolar with superimposed dipolar forces, see later.

A brief study of the relation between form of equilibrium and form of growth (more exactly, final growth form) will be inserted here.

The crystals which are investigated are, almost without exception, the product of a growth process. If the form of equilibrium is not a simple crystalline form, the resulting growth form contains only the slow-growing faces large enough to be visible; the quick-growing faces remain the same size as the same faces in the form of equilibrium, which in general is sub-microscopic. The form of equilibrium here is to be ascribed to the pressure prevailing during the process of growth.

Intermediate stages of growth of rounded single crystal forms provide the possibility of making all equilibrium faces visible<sup>11 14 15</sup> It is, unfortunately, always possible that in the course of this faces also appear which do not belong to the equilibrium form. The appearance of  $\{012\}$  and  $\{111\}$  in Spangenberg's<sup>19</sup> and Neuhaus's<sup>20</sup> experiments on growth with spherical, polished NaCl crystals from aqueous solution may be recalled, for example, although the only form of equilibrium here is  $\{001\}$ .

It is therefore of great importance to develop a thoroughly reliable method for the experimental production of equilibrium forms. This was achieved for the first time for urotropine, following on observations by Kaischew,<sup>17</sup> Honigmann<sup>18</sup> and myself. At low temperatures, at which the transfer of matter takes place almost entirely via the adsorption layer, the growth form, which in the case of urotropine is a rhombododecahedron, re-forms the faces  $\{001\}$  and  $\{112\}$  (see Fig. 5). Specially accurate investigations were carried out at 0° C. (If one subjects the crystal to small fluctuations in temperature the same form appears much more quickly.)

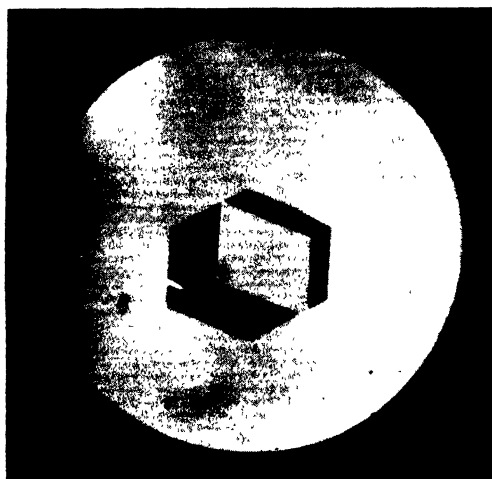
In answer to the question why, up to now, one had neither observed a spontaneous appearance of equilibrium forms of visible size (microscopic), nor considered this possible theoretically, one can say that the relative differences in the vapour pressures of faces of an already visible crystal, which are not in proper ratio to one another, are in fact very small. In spite of this, it is not so much the smallness of the differences of the relative vapour pressure which is responsible for retarding the course of the reaction as Volmer's work of formation of the two-dimensional nucleus connected with the supersaturation. This must appear in the formation of new lattice faces, and as the supersaturation disappears, converges towards infinity. If therefore one succeeds in removing the energy threshold of the work of formation of the two-dimensional nucleus, the process of alteration leading to the equilibrium form on a crystal of the growth form should be possible. It is possible to remove this energy threshold, or to lower it considerably, by the construction of hollow edges starting from which single lattice faces can develop. Only the few crystals whose crystal units show a comparatively high mobility within the adsorption layer at low temperatures will qualify for this.

The discrepancy between theory and experiment, already mentioned, evinces itself with urotropine, by the appearance of  $\{112\}$  of the faces referred to the nearest neighbour but two, but not of  $\{111\}$ . As is to be set forth elsewhere by Honigmann and myself, the experimental result can be explained by the fact that a profound alteration in lattice takes place in the uppermost lattice face of  $\{112\}$ . This is probably a lattice alteration which is also stable at a low temperature.

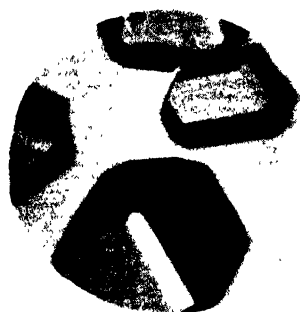
With urotropine another phenomenon can be studied. Certain alterations

<sup>19</sup> Spangenberg, *N. Jahrb. Miner., Mügge-Festbd. A*, 1928, **57**, 1197.

<sup>20</sup> Neuhaus, *Z. Krist.*, 1928, **68**, 15.



(a) Growth form (011).



(b) The form which is formed on tempering (011, 001, 112). (Honigmann).

FIG. 5.—Urotropine crystals.





in the surface lattice do not appear until the temperature is high, i.e., alterations also exist which show the character of two- and three-dimensional changes. Above 170° C the mechanism of growth and evaporation, as well as the form of equilibrium of the urotropine crystal, changes fundamentally.<sup>18</sup> The growth and reduction take place now in multimolecular (visible) layers, whose border is completely rounded; the form of equilibrium is a rhombic dodecahedron whose corners and edges are also rounded.

This phenomenon is obviously connected with the fact that new degrees of freedom (rotations) of crystal units of certain lattice surfaces, edges or peripheral rows are aroused by temperatures considerably lower than those in the inside of the crystal.

In closing, the question may further be asked, how the equilibrium form of a crystal changes when it is surrounded by a liquid instead of its own diluted vapour. The simplest case would be to suspend the small crystals in their own fused liquid.

The specific interface energy of a certain face  $\sigma_{hkl}$  would be given here by the following relation:

$$\sigma_{hkl} = {}_1\sigma + {}_2\sigma_{hkl} - {}_{12}\sigma_{hkl}, \quad (8)$$

where  ${}_1\sigma$  and  ${}_2\sigma_{hkl}$  are the corresponding values for the liquid and the crystal relative to vacuum, and  ${}_{12}\sigma_{hkl}$  the work which would be obtained by the contact of unit areas of crystal and liquid. It is seen that  $\sigma_{hkl}$  is not only very small if  ${}_2\sigma_{hkl}$  is very small, but also when  ${}_{12}\sigma_{hkl}$  is especially large. The latter is all the more likely to be true, the more continuous the transition from crystal via the interface to liquid. The growth form of Cd which is produced from the fused liquid<sup>14,15</sup> may be quoted here as an example. It is seen that the face  $\{11\bar{2}1\}$  appears here, which as a rule is coarsened on continuing to grow in vapour, as it does not belong to the equilibrium form of the crystal surrounded by vapour phase (see Fig. 4).

The general case of an equilibrium form surrounded by a phase of any desired composition has not yet been accurately treated, either experimentally or theoretically. Up to the present a certain amount of attention has only been paid to the occasional growth forms showing great deviations, which precipitate from various solutions.

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## SURFACE STRUCTURES OF IONIC CRYSTALS

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There is no doubt that the structural peculiarities of crystal surfaces play a role in many physical and chemical processes in which the surfaces of solid bodies participate. Up to the present no experimental method exists which is dependable enough for the determination of surface structures. The only way to decide how the positions of equilibrium of the atoms in the surfaces can differ from those of the infinitely extended space lattice is to employ theoretical considerations with the use of simple models.

In the following report is an account of calculations on the model of the rocksalt lattice, the aim of which was to determine the surface structures caused by the effect of the ionic polarizability. Our work\* follows on an investigation by Lennard-Jones and Dent<sup>1</sup> published twenty years ago, but goes further in that not only the displacement of the ions in the direction of the surface normals are taken into consideration, but also the tangential distortion of the surface lattice.

As in the case of Lennard-Jones, besides the electrostatic forces, only forces of repulsion of small range according to Born's power function are taken into account; here also a simplified surface structure is assumed where only the ions of the uppermost lattice face undergo deformation and displacement, while the rest of the lattice below it remains undeformed. Only average ionic properties of the two components are taken into account (except for the signs of the charges), i.e., the potentials of repulsion and the polarizabilities for both kinds of ions are regarded as equal.

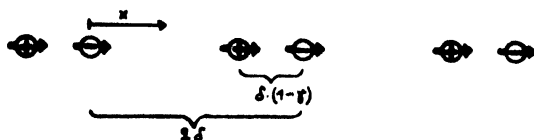


FIG. 1.

The idea directing our calculation was the consideration that the polarizability of the ions can have the effect of lowering the symmetry. This idea was applied by Born and Heisenberg<sup>2</sup> and by Hund<sup>3</sup> to ionic molecules, and by the latter to three-dimensional ionic lattices. If one imagines the polarizability of these to be continuously increased, the co-ordination lattices, which at first are stable, change at a certain polarizability into less symmetrical structures, namely, to layer- or molecule-lattices. These "polarization sub-structures" are distinguished by the fact that the ions prefer those positions where the electrical field has the highest possible intensity, in other words, where the gain in polarization energy is as great as possible.

From the fact that an isolated (001)-lattice face in equilibrium must have a lower lattice constant compared with a compact space lattice (2.68 Å for NaCl, calculated using the function of repulsion which is obtained from the lattice constant 2.81 Å and the compressibility of the space lattice) Lennard-Jones and Dent<sup>1</sup> came to the conclusion in their above-mentioned investigation that a tendency to contract exists in the surface, which they treated as analogous to the surface tension of a liquid. The question of how such a contraction could be brought about was not followed up by Lennard-Jones and Dent; above all, the part played by the ionic polarizability in a tangential deformation of the surface was not considered. We will show that no tendency towards tangential contraction exists in the case of low polarizability in the surface. Only a decrease in the distance between the two uppermost lattice faces is to be expected, similar to that already calculated by Lennard-Jones and Dent. With more highly polarizable ions, on the

<sup>1</sup> Lennard-Jones and Dent, *Proc. Roy. Soc. A*, 1928, **121**, 247; see also Madelung, *Physik. Z.*, 1919, **20**, 494; Zwicky, *Helv. physik. Acta*, 1930, **3**, 269; Stranski, *Jb. Univ. Sofia*, 1927-28, **24**, 297; *Z. physik. Chem.*, 1928, **136**, 259.

<sup>2</sup> Born and Heisenberg, *Z. Physik*, 1924, **23**, 388.

<sup>3</sup> Hund, *Z. Physik*, 1925, **34**, 833.

\* An account of our calculations which have been supplemented in the meantime by further results has already been published in *Z. Physik*, 1948, **124**, 421 and 429.

other hand, tangentially deformed surface structures are favoured from the standpoint of energy. These are also adapted to the periodicity of the space lattice situated below, but in such a way that adjacent ions collect together to form small isolated molecular complexes.\* That is to be seen best from a consideration of one- and two-dimensional structures.

**One- and Two-dimensional Lattices.**—The investigations on stability carried out by Hund<sup>3</sup> in three dimensions, but only approximately, can easily be calculated exactly for one- or two-dimensional lattices. How will a chain consisting of alternate positive and negative ions and an isolated lattice face of the rocksalt (001)-type behave respectively by a continual rise in the ionic polarizability? Certain limiting assumptions must first be made concerning the form of the sub-structures of low symmetry which result from the co-ordination structures, and which one can imagine as being produced from the first by a homogeneous deformation. The chain or lattice face will break up into single insular complexes, and it seems plausible to expect that similar crystal units in the structure thus formed will assume equivalent positions as regards energy and structure and that the ionic complexes formed are electrically neutral. Thus, for the alternating chain only a division into double ion molecules comes into question, whilst for the lattice face four-ion insular complexes are conceivable too. Furthermore, "chain lattices" can also be formed here (analogous to the layer lattices in three dimensions), i.e., the complexes formed can extend over the whole surface in simple co-ordinative relationship. If one introduces the additional assumption that the complexes formed by a division of the original co-ordination lattice in the manner suggested are deformed by the effect of the polarization forces with no loss in their own symmetry, one is bound to arrive, in the case of the (001)-lattice face, at the types shown in Fig. 2. These structures may be characterized, as indicated in the figures, by means of a relative parameter  $\gamma$  (the relative approach between immediate neighbours, referred to the distance between the ions  $\delta$  in the undeformed structure). The lattice energy of the chain or lattice face must now be formulated as a function of  $\gamma$ .

In addition to the coulomb ionic effect and the energy of repulsion we introduce the polarization energy for one ion in the form

$$-\vec{p} \cdot \vec{E} + \vec{p}^2/2\alpha$$

where  $\vec{p}$  is the dipole moment produced in the single ion;  $\vec{E}$  is the "self-field intensity" arising from all other charges and dipoles;  $\alpha$  is the mean polarizability. The components of  $\vec{p}$  can be eliminated by putting the partial differential quotients equal to zero as was done by Born and Heisenberg.<sup>2</sup> The following equation† is then obtained for the lattice energy for an ion pair:

$$-U = \frac{e^2}{\delta} \left\{ V^{(p)} + \frac{F_s^{(p)^2}}{\delta^3/\alpha - F_s^{(d)}} \right\} - \frac{\Lambda}{\delta^n} V^{(B)}.$$

$e$  is here the elementary charge,  $\Lambda \cdot r^{-n}$  the repulsion potential of two ions (we use  $n = 9$ );  $V^{(B)}$  is Born's repulsion potential,  $V^{(p)}$ ,  $F^{(p)}$ ,  $F^{(d)}$  self-potentials or self-field intensities for corresponding forms with the lattice constant  $\delta = 1$ , originating from the poles ( $p$ ) and dipoles ( $d$ ). These

\* A similar hypothesis was already put forward by one of us in 1928.

† The easily proved rule must be taken into account that for all configurations of the type under consideration the mutual effect between one dipole and all other ionic charges is equal in value to that between one ionic charge and all other dipole moments.

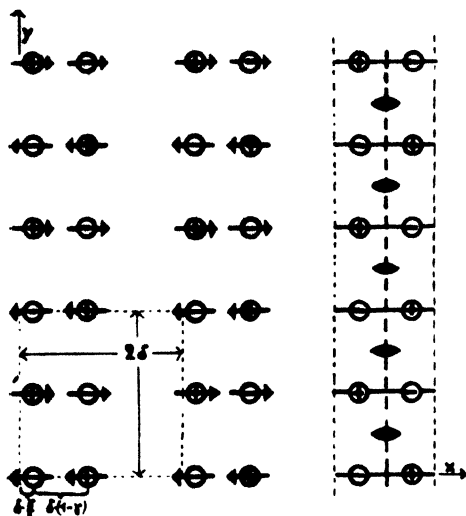


FIG. 2 a.

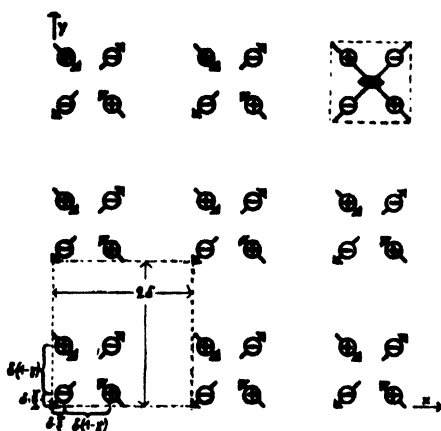


FIG. 2 b.

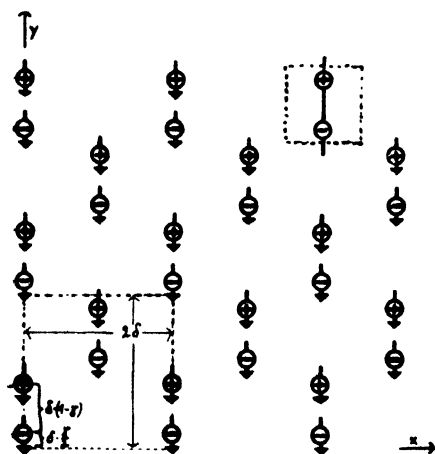


FIG. 2 c.

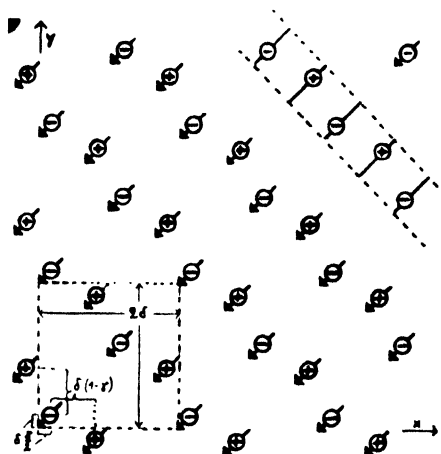


FIG. 2 d.

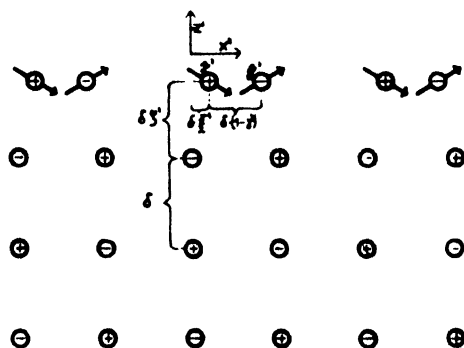


FIG. 3.

values are functions of  $\gamma$ , and can be calculated according to the well-known methods of Madelung<sup>4</sup> and Ewald.<sup>5</sup>

From the condition

$$\partial^2 U / \partial \gamma^2 = 0,$$

one can find the "critical polarizability" above which the tangentially deformed structures must be more stable than the co-ordination structures. If, for the lattice constants of the latter, one inserts the equilibrium values of  $\delta = 2.49 \text{ \AA}$  for the chain and  $\delta = 2.68 \text{ \AA}$  for the lattice face, one obtains :

$$\begin{array}{lll} \text{for the chain} & \dots & C_{\alpha_{2.49}}^{\text{crit.}} = 1.82 \text{ \AA}^3; \\ \text{for the lattice face} & \dots & F_{\alpha_{2.68}}^{\text{crit.}} = 1.95 \text{ \AA}^3. \end{array}$$

But if one stretches the chain or lattice face under force, so that their lattice constant adjusts itself to the value  $\delta = 2.81 \text{ \AA}$  of the compact space lattice,  $\alpha^{\text{crit.}}$  becomes much smaller, namely :

$$\begin{array}{lll} \text{for the chain} & \dots & C_{\alpha_{2.81}}^{\text{crit.}} = 0.58 \text{ \AA}^3; \\ \text{for the lattice face} & \dots & F_{\alpha_{2.81}}^{\text{crit.}} = 1.50 \text{ \AA}^3. \end{array}$$

The mean value of the polarizabilities of  $\text{Na}^+$  and  $\text{Cl}^-$  amounts to  $\bar{\alpha} = 1.61 \text{ \AA}$ . The function  $U(\gamma)$  for this value of  $\alpha$  and for  $\delta = 2.81 \text{ \AA}$  was plotted graphically. It shows an energy minimum :

$$\begin{array}{lll} \text{for the chain at} & \dots & \gamma = 20 \% ; \\ \text{for the lattice face at} & \dots & \gamma = 6 \% . \end{array}$$

For the (001)-lattice face the structure type (a), the chain lattice parallel to the edge, proves to be the most stable. The figures quoted refer to this type. If one puts the lattice face or edge as the surface in a space lattice, the deformation of the surface is diminished through the effect of the undeformed remaining part of the lattice, as is shown below.

**The (001)-Surface.**—The expression for the lattice energy of the lattice face must now be completed by the terms which express the potential energy of the ions of the uppermost lattice face in the field of the half-lattice lying below, which is undistorted and infinitely extended.\* The field intensity where the surface ions are situated now has components perpendicular to the surface ( $z$ -direction). The lattice energy depends therefore upon a further parameter  $\zeta$ , for which we choose the relative distance (referred to  $\delta$ ) between the two uppermost lattice faces (see Fig. 3).

The lattice energy per pair of ions —  $U_{(001)}$  is now

$$\frac{e^2}{\delta} \left\{ ({}^F V^{(p)} + 2{}^H V) + \frac{({}^F F_x^{(p)} + {}^H F_x)^2}{\delta^3/\alpha - {}^F F_x^{(d)}} + \frac{{}^H F_z^2}{\delta^3/\alpha - {}^F F_z^{(d)}} \right\} - \frac{\Lambda}{\delta^n} [{}^F V^{(B)} + 2{}^H V^{(B)}].$$

The values  ${}^F V$ ,  ${}^F F$  represent the self-potentials and self-field intensities of the surface already introduced above,  ${}^H V$ ,  ${}^H F$  the potentials and field intensities induced by the ions of the half-lattice at the points where the surface ions are situated; these are defined, as previously, for lattices with a distance between the ions of 1. They can be calculated best according to Madelung's<sup>4</sup> method.

<sup>4</sup> Madelung, *Physik. Z.*, 1918, **19**, 524.

<sup>5</sup> Ewald, *Ann. Physik*, 1921, **64**, 253.

\* These terms, of course, must be substituted in the expression for the lattice energy of the uppermost lattice face in their full amounts (or if one refers to a pair of ions, with the factor 2), and must not be halved like the self-mutual effect of the surface ions. This fact was overlooked by Lennard-Jones and Dent.<sup>1</sup>

As the direct application of the conditions of equilibrium

$$\partial U / \partial \gamma = 0 \text{ and } \partial U / \partial \zeta = 0$$

is too complicated, we determine the minimum potential energy (maximum lattice energy) by a graphical method. If  $(-U)$  for certain values of the tangential parameter  $\gamma$  is plotted against the vertical distortion co-ordinate  $\zeta$ , curves are obtained of the type in Fig. 4 *a*. The figure refers to the type (a) (Fig. 2), the chain lattice parallel to the edge, and the polarizability

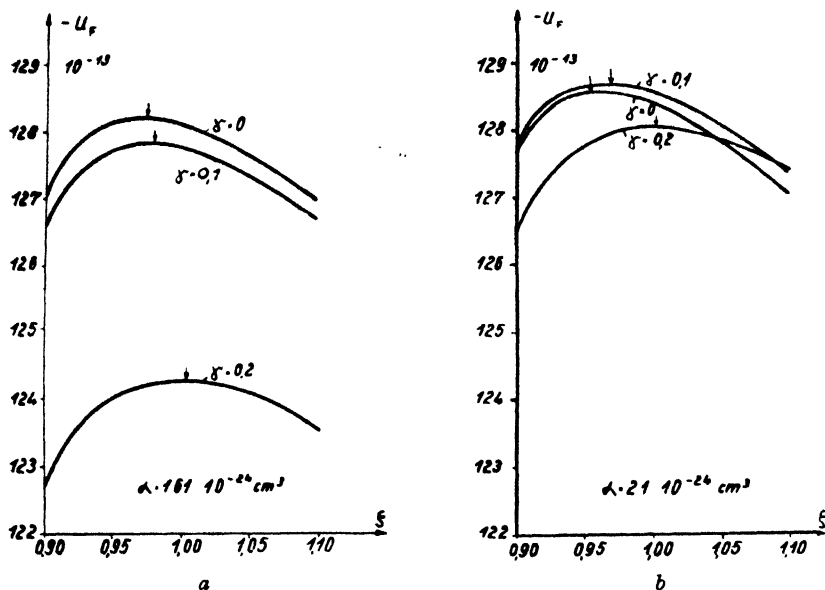


FIG. 4.

$\alpha = 1.61 \text{ \AA}^3$ . The curve  $\gamma = 0$  shows a maximum at  $\zeta = 0.975$ , which corresponds to a decrease in the distance between the uppermost lattice faces of 2.5 % (according to Lennard-Jones and Dent, 5 %). As the tangential distortion increases, the maxima move towards the right, the contraction thus becomes still smaller.

TABLE I

Type	Equilibrium value of	
	$\gamma$	$\zeta$
<i>a</i>	0.11	0.97
<i>b</i>	0.08	0.97
<i>c</i>	0.05	0.96
<i>d</i>	0.01	0.96

With a somewhat higher value for  $\alpha$  at  $\gamma$ -values differing from zero a flat maximum is soon formed, and as the polarizability further increases this moves to the right and becomes steeper. Fig. 4 *b* and 5 *b* show the corresponding curves for  $\alpha = 2.1 \text{ \AA}^3$ . The maxima lie at values for the parameter given in Table I.

The greatest tangential distortion and lowest energy is to be found for type (a), the chain lattice parallel to the edge. Thus this would seem to be the

In Fig. 5 *a* the maximum ordinate values of Fig. 4 are represented as a function of  $\gamma$ . In the same figure are the corresponding curves for the types (b), (c) and (d). It can be seen that for the ionic polarizability of NaCl ( $\alpha = 1.61 \text{ \AA}^3$ ), all the maxima lie at  $\gamma = 0$ , i.e., the lattice face retains its complete symmetry. At the most, the maximum for type (a) could lie somewhat to the right of the position  $\gamma = 0$ .

predominating polarization sub-structure found for the (001)-face. Then follow the types (b) and (c) and with a greater difference the diagonal chain lattice (d).

From the result one can conclude that for NaCl probably no decrease in the symmetry of the face is to be expected on the cube faces. This should only set in with higher polarizability.

**The [001]-Edge.**—It is in line with the approximate method used up to now to deform independently only the ion chain which forms the edge of the crystal, whilst all inner atoms of the crystal quadrant remain unpolarized in their normal positions. Those structures in the surface lattice faces adjacent to the edge are to be fixed which, according to the calculation above, represent the configurations of minimum energy for the infinitely extended surface. In this case also we allow the whole row on the edge to move its position relative to the rest of the crystal, confining ourselves to a displacement in the plan which divides the angle between the adjacent cube faces.

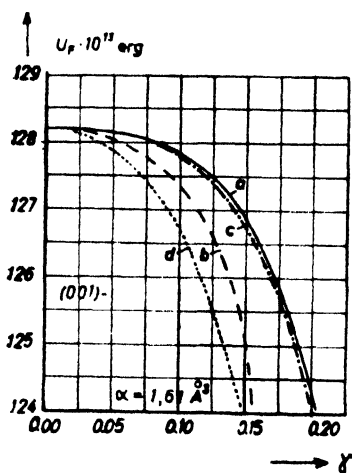


FIG. 5 a.

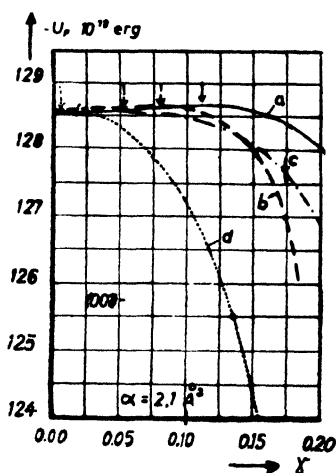


FIG. 5 b.

According to the previous section, when the polarizability is low the faces remain undistorted tangentially, their ions are polarized perpendicularly to the face and the distance between the two uppermost lattice faces is somewhat decreased. In this case the critical polarizability for a conversion of the edge to a linear molecular lattice amounts to  $\alpha_{\text{crit.}} = 1.4 \text{ Å}^3$ . If one substitutes the polarizability  $1.61 \text{ Å}^3$  (corresponding to NaCl) the energy minimum then lies at  $\gamma = 0.04$ , which indicates a 4 % approach between neighbouring ions. The edge is displaced about 5 % in the direction of the remaining crystal quadrant.

If we now use the higher polarizability of  $2.1 \text{ Å}^3$ , we must assume the chain lattice structure type (a) (see Fig. 2) in the cube surfaces. As the chain structure can take up different positions relative to the edge, different types of combinations must be considered, from which we pick out the three types in Fig. 6 as those most favoured from the standpoint of energy. According to the calculation carried out, type ( $\alpha$ ), in which the chains run vertically to the edge on both sides, is found to be the most stable. A molecular structure is obtained in the edge with an 18 % approach between the nearest neighbours (compared with 11 % in the adjacent



faces). In equilibrium, the whole edge alters its position relative to the rest of the crystal by 4 % (compared with 2.5 % for the face).

**The Surface Structures for Crystals of the Rocksalt Type.**—The formulæ for the lattice energy of faces and edges contain the polarizability in the combination  $\alpha/\delta^3$  only. If one makes the assumption (somewhat rough, of course) that the repulsion potentials for all lattices of the rocksalt type can be represented by means of a power function with the same repulsion exponent ( $n = 9$ ),  $\Lambda/\delta^{n-1}$  is a common constant for all lattices. ( $\Lambda$  is calculated from the equilibrium lattice constant of the space lattice which is obtained experimentally.) Thus the value  $\delta.U$  is a pure function of the quotient  $\alpha/\delta^3$ . It is thus now possible to specify critical values for this quotient, which are determining factors in a sub-structure formation in the faces and edges. These are

$$(\alpha/\delta^3)_{\text{crit.}} = 0.064 \text{ for the } [001]\text{-edge};$$

$$(\alpha/\delta^3)_{\text{crit.}} = 0.073 \text{ for the } (001)\text{-face.}$$

No decrease in symmetry in the faces and edges is to be expected in the alkaline halides for :

Salt	KF	NaF	RbF	CsF	KCl	LiF
$\alpha/\delta^3$	0.047	0.047	0.056	0.061	0.062	0.063

The cube faces retain their full symmetry, whilst the edges show molecular structure for the salts :

Salt	RbCl	KBr	RbBr	NaCl
$\alpha/\delta^3$	0.065	0.069	0.070	0.073

The edges have strongly defined molecular structures, the faces exhibit chain lattice structures in the following :

Salt	RbI	KI	NaBr	LiCl	NaI	LiBr	LiI
$\alpha/\delta^3$	0.079	0.080	0.082	0.091	0.096	0.101	0.117

The way in which the lattice energy behaves at the polarizability  $\alpha = 2.1 \text{ \AA}^3$  shown above in Fig. 4 and 5 might fit the case NaI.

The anion is the chief determining factor for the values of the mean polarizability. On the other hand, the denominator of the quotient  $\alpha/\delta^3$  is the lattice constant  $\delta$ , which has especially low values for salts with small (strongly polarizing) cations. Therefore, the most strongly defined polarization sub-structures in the surfaces are to be expected in salts with large anions and small cations.

**Decrease in Symmetry in Individual Faces.**—It must be assumed that the decrease in symmetry in individual faces will show itself externally in some way in the physical properties of the faces. No direct method for the determination of surface structures exists, however, as yet ; it is known that when electron diffraction is brought about, space lattice regions of considerable thickness are always involved. Clues to a decrease in symmetry in the surface are given by numerous experiments using the etching method,<sup>6</sup> in which a lower symmetry actually was found than that corresponding to the space lattice. But up to the present, no clear connection

<sup>6</sup> Brauns, *N. Jahrb. Miner.*, 1886, **1**, 224 ; 1889, **1**, 121. Rosicky, *N. Jahrb. Miner.*, 1916, **2**, 15.

with our theory of polarization sub-structures could be established, as in the etching method apparently there are too many unpredictable conditions playing a part.

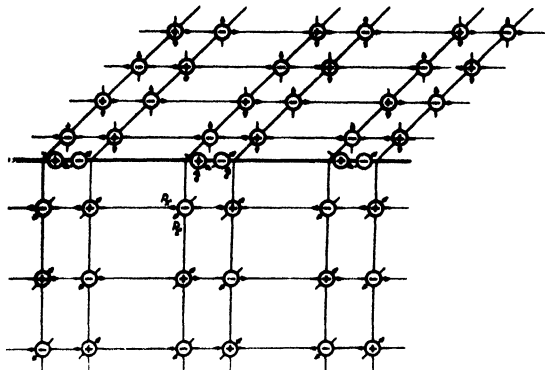


FIG. 6 a.

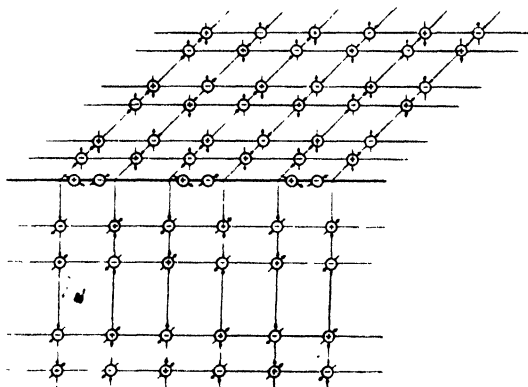


FIG. 6 b.

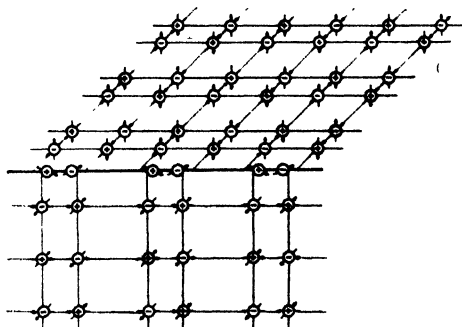


FIG. 6 a/b.

### Reorientation Processes ; Decrease of Symmetry of the Habitus.—

It can be seen from Fig. 5 that the different sub-structure types of the (001)-face are in fairly keen competition with one another from the standpoint of energy (ordinate scale  $10^{-13}$  erg/ion pair). One must further note that each type of structure possesses four possible forms (e.g., orientation of the chain lattice in the  $x$ - and  $y$ -direction). It must be assumed that spontaneous reorientations take place between the different structures.

It is possible that, as co-operative processes, such changes would require considerable energy of nucleus formation or activation. As a result the frequency of such reorientation processes would be dependent upon temperature.

As the molecular structures are especially well defined on the edges, as was shown above, there should be the fewest reorientation processes taking place there. Thus the edges function as nucleus-forming centres, i.e., they determine the structures of the adjacent parts of the faces, and the most favoured are chain lattice structures of the type ( $\alpha$ ) (Fig. 6 a) with the chain running perpendicular to the edge.

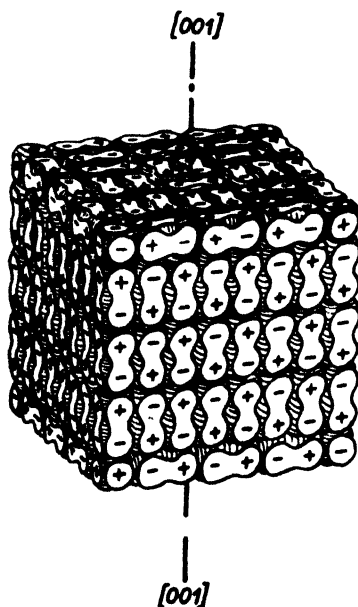


FIG. 7.

If one considers the complete cubic crystal-body, it is conceivable that parallel sub-structures will be formed on the cube faces of a zone through mutual influence across the common edge. Such a case is shown diagrammatically for the zone  $\{001\}$  in Fig. 7. According to the temperature, a more or less frequent change in structure will set in on the two faces which do not belong to the zone, and this can also lead to occasional reorientation of the complete zone structure beyond the edge, but on the whole the parallel surface structures of the faces of the zone in question would be especially stable. The difference in the degree of orientation of the two faces could perhaps show itself in their growing and adsorption properties, by which a lower symmetry of the whole crystal body (crystal habitus), in this case a tetragonal form, could be simulated.

Nothing like this is known up to the present for the rocksalt lattice, but an analogous case might be found in the cubic space-centred lattice of crystallized ammonia. The growth form here is the presence of pectic acid, crystallization in

rhombic dodecahedron. In the long needles <sup>7</sup> was observed.

**Influence of External Forces ; Tensile Strength.**<sup>8</sup>—There is no doubt that surface structures are sensitive to the effect of external forces, in which, incidentally, one may include the forces which proceed from an adsorption layer or a neighbouring phase, mentioned in the previous example. Tangential electric fields, for example, would probably favour the structure types (c) and (d) of Fig. 2 which possess a tangential electric moment, from the standpoint of energy. This might express itself in dielectric or optical anomalies, unless the effects are too small to be observed.

If one submits the crystal to a tensile force in the direction of a cube edge, the tendency to form molecular lattice structures in the edges and faces running parallel to the direction of the force will increase greatly with only a small stretch. Molecular cracks, perpendicular to the direction of the force, are thus produced in the surface. It is conceivable that at a certain tensile force, which lies far below the theoretical tensile strength

<sup>7</sup> Ehrlich, *Z. anorg. Chem.*, 1932, **203**, 26.

<sup>8</sup> Stranski, *Ber.*, 1942, **75**, 1667.

of the infinitely extended crystal, these surface cracks will extend further into the inside of the crystal. The cracks produced would coincide with the breaking-faces of the crystal. Thus the presence of atomic sharp edges would be a decisive factor for the lowering of the tensile strength, compared with the theoretical value, as is actually confirmed experimentally. The experimental data of the Joffé effect<sup>9</sup> are in agreement with this, namely,

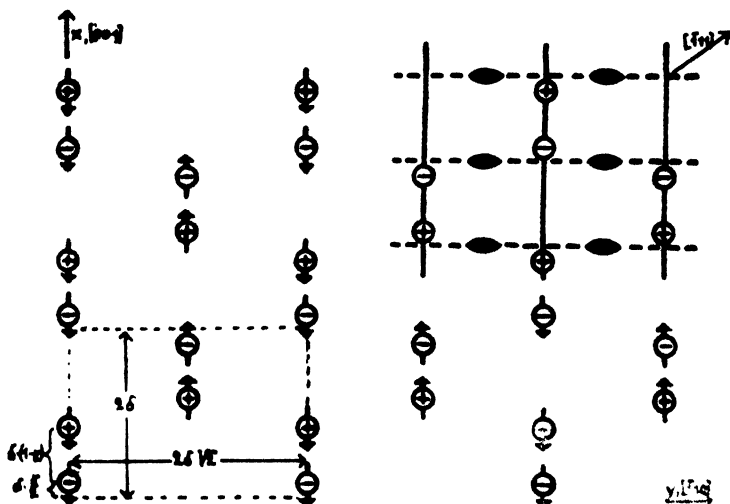


FIG. 8 a.

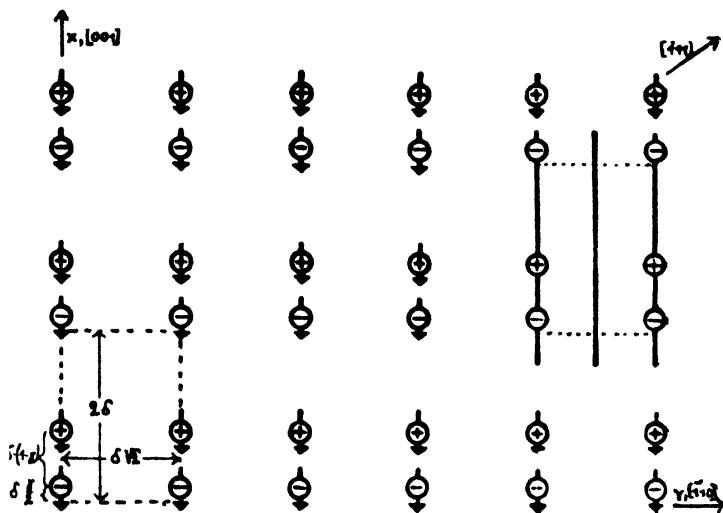


FIG. 8 b.

if the edges are removed by superficial solution of the crystal, the tensile strength (that is, its upper limit in a great number of experiments) increases until almost at the theoretical value. With a renewed growing process (new formation of the atomic sharp edges) the tensile strength again greatly decreases.

<sup>9</sup> Joffé, Kirpitschewa and Lewitzky, *Z. Physik*, 1924, 22, 286.

By carrying out the necessary calculations, we have convinced ourselves how the surface structure of a superficially dissolved crystal might be expected to behave. This is known to consist of numerous atomic cube face steps. It was found that the ion chain which forms the steps does not exercise a directing influence upon the chain structures of the two adjacent parts of the face, as does the edge. The crack structures produced in the face are isolated to a certain extent by the steps. This result could be supported by means of calculations on the equilibrium structure of the (011)-face. This might be taken as representative for the structure of the superficially dissolved surface, as it is made up exclusively of atomic cube steps. The only sub-structure types of the (011)-face which come into question are shown in Fig. 8 *a, b*. The calculation of the lattice energy shows that the type A is  $2 \times 10^{-14}$  erg/ion pair more stable than type B. The distortion parameter (defined according to Fig. 8) amounts in equilibrium to 8 % for NaCl, 20 % for NaI. Thus for the (011)-face it would seem that surface structures will be chiefly formed in which the molecular surface cracks do not lie in the track of possible breaking surfaces, as is the case in type B. In the more stable type A, on the other hand, the surface cracks follow  $[\bar{1}\bar{1}1]$ , i.e., in the track of rhombic dodecahedron faces; these are possible sliding-faces of the crystal. This could explain the increase in plasticity of superficially dissolved crystals.

From our considerations, however, we are not able to produce a mathematical theory for the cracking. Among other things, the influence of statistically distributed lattice disturbances would have to be included. But it seems certain to us that structural irregularities in the surface will have to be taken into consideration in any exact theory of the future.

**Considerations Concerning the Justification of the Assumptions Made.**—One objection which could be made to our way of calculating refers to the use of the linear polarization expression,

$$\overset{\rightarrow}{p} = \alpha \overset{\rightarrow}{E}.$$

This is known to be valid for homogeneous fields and small field intensities only. It is certain that neither of these conditions are fulfilled in crystal surfaces.

Estimates as regards energy, which refer to alkaline halide molecules,<sup>10</sup> give cause for the assumption that, in reality, the share of the polarization energy in the total bond energy is considerably greater than the share calculated from the polarizability in the homogeneous field. If one subtracts the energy of repulsion, calculated from crystal lattice data, from the energy of dissociation known from spectroscopic data, and makes a correction for the effect of van der Waals's forces, the remainder is more than twice as great as the classic polarization energy. The term remaining contains the quantum-mechanical mutual effect of the electron-clouds, which is difficult to estimate, but it can scarcely be assumed that this is very great. It can therefore be assumed that in the mutual effect of the ions in a crystal surface also the polarization share is still greater than that calculated by us according to the classical method. The data which we give for the surface distortions probably represent, therefore, a lower limit of the structural deviations realized in nature.

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<sup>10</sup> Hellmann and Pschejtzkij, *Acta Physicochim.*, 1937, 7, 621.

# CRYSTAL GROWTH AND SURFACE STRUCTURE

## Part I

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**Introduction.** As a preliminary to the study of the rate of growth of crystals, we consider in Part I of this paper the influence of surface structure on the rate of advance of the growing surface. If, for the time being, we confine our attention to crystals with perfect lattices, it is found that crystal surfaces can be divided into two classes, (a) close-packed † surfaces and (b) non-close-packed or "stepped" surfaces, which possess essentially different properties. A surface is close-packed if, when it is as flat as possible, all the surface molecules are at the same distance from a plane parallel to it; in all other cases the surface will present a stepped appearance, as in Fig. 1, the height of each step being of molecular dimensions. By way of illustration, in the simple cubic system (100) surfaces, (111) surfaces and (110) surfaces are close-packed, all other surfaces are stepped. In a stepped surface

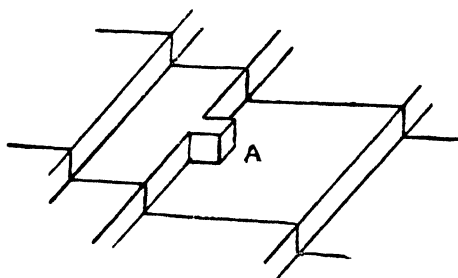


FIG. 1.

the terraces in Fig. 1 are pieces of close-packed surface. In the case of a (110) surface of a simple cubic crystal, the terraces will be (100) surfaces. This point of view is fruitful because it can be shown that for a stepped surface the portions between the steps play almost no part in growth phenomena at low supersaturations. In fact, deposition on a close-packed surface can only take place by surface nucleation: small "islands" of molecules collect on the surface and grow so as to produce a new layer, a process which is very slow at low supersaturations. On the other hand, deposition on the edge of a step A (Fig. 1) can take place without there being a linear nucleation process. Hence the growth problem for a stepped surface is essentially solved, once the corresponding problem for steps has been solved. If a crystal grows at all, some kind of steps must exist at some time in the surface. These steps may be of the kind already mentioned, or they may be boundaries of two-dimensional nuclei. Growth essentially depends on the existence of "kinks" in these steps. Easy growth is guaranteed if these

\* Seconded from I.C.I., Ltd., Butterwick Research Laboratories, The Frythe, Welwyn, Herts.

† Note that our definition of "close-packed" differs somewhat from current usage.

kinks are always present, and this criterion can be reduced to the question of the existence of kinks, when the external concentration is the equilibrium value. For if kinks are present at equilibrium, then when the external concentration is raised there are already suitable deposition points available. If there are no kinks at equilibrium, then these must be created, and a large hindrance to growth appears. It can be shown that the concentration of kinks in a step in equilibrium is high, and that the concentration of kinks in a close-packed surface at equilibrium is negligible. This again speaks in favour of our classification of surfaces into stepped and close-packed surfaces.

In this paper we are concerned with the equilibrium structure and rate of growth of an infinite surface. It is, of course, clear that an infinite surface is in equilibrium with the same external concentration (e.g., vapour concentration) whatever the surface. A finite surface will not be in equilibrium in the same sense as in the case of an infinite surface, and consequently, in general, some change will tend to take place. But changes of orientation can take place only by means of processes which occur at the boundary of the surface, and hence for a surface of observable size the change will occur at an unobservably slow rate, the associated relaxation time tending to infinity with the size of the crystal. Therefore, if we confine ourselves to a region on a finite surface which is almost flat, then its structure will be the same as that in an infinite surface having the same orientation.

The two basic equilibrium problems are now (a) the equilibrium structure of an infinite step, and (b) the equilibrium structure of an infinite close-packed surface.

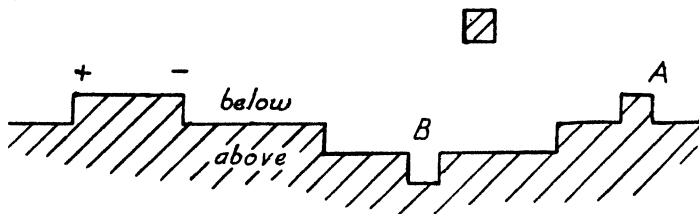


FIG. 2.

**The Infinite Step.** Consider then, a semi-infinite layer of molecules on an infinite close-packed plane crystal surface bounded by a connected line. This we call a step: it can have any mean direction. At  $T = 0^\circ \text{K}$ , the step will be perfectly straight, but as the temperature is increased it will consist of a number of "kinks," separated by certain distances as in Fig. 2, a certain number of adsorbed molecules (A) and a certain number of holes (B). We need to know only the concentration of kinks to form a picture of the mean structure of the step. This idea, introduced by Frenkel<sup>1</sup> simplifies the treatment of the problem very much: we call the kinks "Frenkel kinks" (F.k.). It is clear that the concentration of kinks in a step will depend on its orientation, and that there will be orientations for which the number of kinks is a minimum. For instance, for the (001) face of a simple cubic crystal, the (10) steps will have the smallest number of kinks. This minimum number will tend to zero with  $T$ . Accordingly, if we can show that a (10) step contains a large number of kinks at  $T > 0$  under equilibrium conditions we know that steps of all orientations also contain a large number of kinks.

If we use a simple cubic model with nearest neighbour interactions (Kossel crystal) it is easy to find the equilibrium concentration of adsorbed molecules,

<sup>1</sup> Frenkel, *J. Physics, U.S.S.R.*, 1945, 9, 392.

holes and kinks in a (10) step. Let the energy of interaction between neighbouring molecules be  $\varphi$ . Then the energy necessary to form an adsorbed atom in the step (Fig. 3) will be  $\varphi$ . The energy to form a hole is also  $\varphi$ , since an energy  $2\varphi$  is required to form a hole and an adsorbed molecule. The energy to form a kink is, however, only  $\frac{1}{2}\varphi$ , since from Fig. 4 and Fig. 5 only an energy  $2\varphi$  is required to form four kinks. There is no change in energy in going from Fig. 4 to Fig. 5. The numbers of positive and negative kinks (Fig. 2) are, of course, equal. We conclude that the probability for having a hole or an adsorbed molecule at a given place on the step are both given by

$$n = \exp(-\varphi/kT) \quad (1)$$

and that the probability for having a kink at a given place on the step is given by

$$n_+ = n_- = \exp(-\varphi/2kT) \quad (2)$$

If  $T \sim 600^\circ \text{K}$  and we take a typical value of  $\varphi$  as  $0.2 \text{ eV}$ , we find that there is a kink for every ten molecules in the step, and an adsorbed molecule or hole for every hundred molecules.

We have, of course, simplified the problem very much: there is a considerable probability for the existence of kinks of multiple height, particularly for steps which deviate a great deal from the [10] direction (in the simple cubic case). The complete theory has been developed elsewhere.<sup>2</sup>

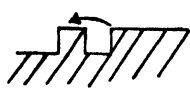


FIG. 3.



FIG. 4.



FIG. 5.

In view of the fact that steps of different orientations have different concentrations of kinks in them, the edge-free energy per unit length of a step varies with the orientation and is a minimum for the (10) step. It might be thought that steps other than (10) steps are not in real equilibrium and that there must be a tendency for these steps to change into (10) steps. If we are considering infinite steps, this conclusion would be erroneous, for it can be shown that steps of all orientations are in equilibrium with the same external concentration of molecules, despite the fact that the concentration of Frenkel kinks varies with the orientation.

Frenkel<sup>1</sup> has treated the kinetical problem of the transformation of any step into a (10) step, assuming on energy grounds that the others are not in equilibrium. He obtains in this way a time of relaxation independent of the length of the step. This result is incorrect because the "torque" which applies to the steps not in a [10] direction is evaluated by Frenkel by taking the derivative of the potential energy with respect to the orientation angle. But his formula contains only points corresponding to the equilibrium positions of the steps, and not the intermediate non-equilibrium positions through which the step would have to pass in order to change at all. In fact for an infinite step, each equilibrium position is surrounded by infinitely high potential barriers which cannot be surmounted.

For a finite step, the situation is different. Such a step can only be in equilibrium with a supersaturated or undersaturated external phase, and then the equilibrium is unstable and subject to stringent restrictions as regards shape. The sharper the corners of a finite step, the greater the

<sup>2</sup> Burton and Cabrera (to be published elsewhere).



rate of evaporation, and an arbitrarily oriented step tends to become a (10) step during the evaporation. The time required for this process to take place increases with the length of the step, because the processes which permit the transformation occur only at the corners.

It must not be thought, however, that all the considerations which apply to kinks in a step apply to steps in a surface. It is still true that surfaces of all orientations are in equilibrium with the same vapour concentration in the same sense as for steps, but the fact that for some surfaces the concentration of steps is large does not imply that double steps, treble steps, etc., will be frequent.\* The difference between the energy of two single steps and one double step is proportional to the step length, and is very large if the interactions are not of the nearest neighbour type. Similarly, there is no question of steps being formed by thermal fluctuations,\* as kinks are formed in a step, since the energy of formation of a step is proportional to its length and is enormous for long steps. Thus a stepped surface tends to become as flat as possible, and at equilibrium, only single steps will appear. It follows that the macroscopic steps which have been observed, e.g., on metals by Graf,<sup>3</sup> on growing crystals have nothing to do with equilibrium problems, but are essentially kinetic in origin. If a surface is produced with macroscopic steps in it, it is obvious that the rate of approach to macroscopic equilibrium is negligibly small and the structure is essentially frozen in.

**Close-packed Surfaces.** The circumstance which makes stepped surfaces so easy to treat is that the steps themselves present a one-dimensional problem. In each position on the step we have a variety of possible states: occupation by a kink of positive, negative or zero height. Each of these possible states can occur independently at each point, and hence the probability for the occurrence of a compound state affecting more than one position is the product of the probabilities for the individual states at each of the individual positions. We have assumed so far that those parts of the crystal surface between steps can be ignored, and this assumption is shown to be reasonable in the following discussion. However, if there are no steps in the surface, which is the normal case in a close-packed perfect crystal surface, then we are presented with an essentially different two-dimensional problem. We assume that in the close-packed surface of a crystal there can be differences of level, i.e., that "jumps" can occur in the surface. The presence of jumps provide suitable places for evaporation and condensation, provided that the jumps are not due merely to the presence of adsorbed molecules and holes. The problem is to estimate the number of jumps at equilibrium as a function of temperature.

In this case the jumps themselves cannot be assigned independently, since it is possible to have twice as many jumps in a surface as there are molecules. Fig. 6 shows a picture of part of a surface; the small squares represent molecules seen from above. The heights of these molecules above some arbitrary plane can, of course, be assigned independently, but the distribution of jumps across the full lines in the figure cannot. For suppose we start at the molecule A and follow any closed path such as ABCDEF, then although we can have any jump we choose between neighbouring molecules on this path, providing we do not close it, the necessity for finishing at A at the same level at which we started implies that the magnitude of any jump on a closed path must be fixed by the magnitude of the others.

<sup>3</sup> Graf, *Z. Elektrochem.*, 1942, **48**, 181.

\* At least, if the interactions are all attractive.

So there are innumerable sets of relations, corresponding to all the closed paths on the crystal surface, between the jump probabilities. In fact, to specify the probability for the existence of a jump at a given point involves the knowledge of the state of the surface at every other point. So we are faced with a so-called co-operative phenomenon. This makes the Frenkel kink picture employed previously almost unworkable. We must therefore look for some other method.

We have made preliminary calculations on the basis of a model which is somewhat oversimplified: we suppose the levels in the crystal surface to be capable of two values only. The method employed is that due to Montroll,<sup>4</sup> Kramers and Wannier,<sup>5</sup> Onsager,<sup>6</sup> Onsager and Kaufman<sup>7</sup> and Wannier,<sup>8</sup> originally devised for the treatment of ferromagnetism, using the two-dimensional Ising model. Just as there is a transition or critical temperature associated with an infinite specific heat in the case of the two-dimensional ferromagnet, so there is in the case of this crystal surface model.

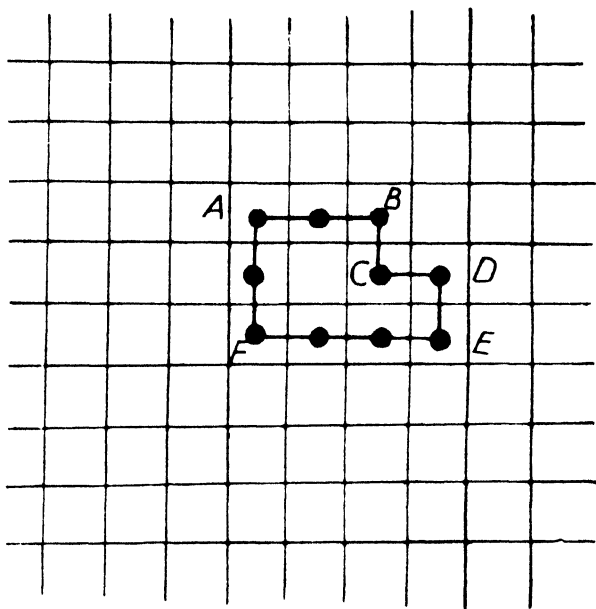


FIG. 6.

The problem is to find the increase in potential energy of the surface due to the presence of jumps in it. The mean number of jumps can then be found. If the surface of the crystal were perfectly flat we should say that the surface potential energy, for example, of the (100) surface of the Kossel crystal per molecule was  $\phi_1/2$  in the nearest neighbour model, corresponding to one unused "bond" per molecule, which we can imagine as sticking out perpendicular to the crystal surface. If, however, the surface is not flat, then there will be additional unused bonds sticking out parallel to the surface, and each of these bonds will contribute  $\phi_1/2$  to the potential energy

<sup>4</sup> Montroll, *J. Chem. Physics*, 1941, **9**, 706.

<sup>5</sup> Kramers and Wannier, *Physic. Rev.*, 1941, **60**, 252, 263.

<sup>6</sup> Onsager, *Physic. Rev.*, 1944, **65**, 117.

<sup>7</sup> Onsager and Kaufman, *Report Int. Conf. on Fund. Particles and Low Temperatures* (Cambridge, July, 1946), Vol. II.: Low Temperatures, Physical Society (1947).

<sup>8</sup> Wannier, *Rev. Mod. Physics*, 1945, **17**, 50.

of the surface. If we take our zero of energy to correspond to a flat surface, then if we evaluate the potential energy per molecule of the surface at equilibrium and divide it by  $\phi_1/2$  we get a figure for the number of unused bonds in the surface which are parallel to it. This figure,  $s$ , represents the "roughness" of the surface. This is the quantity we aim to evaluate as a function of temperature. We expect it to go from 0 to 1 as  $T$  goes from 0 to  $\infty$ .

The crystal surface model we are considering is equivalent to a square lattice of units which we call atoms capable of two states which we designate by  $+1$  and  $-1$ . If two neighbouring atoms have the same state their interaction energy is zero, otherwise it is  $\phi_1/2$ . The first possibility describes the two molecules in the crystal surface when they are at the same level, the second when their levels are different. For the sake of generality we assume, following Onsager, that the interactions can be different in the two directions  $[10]$  and  $[01]$ : in the case of the  $(100)$  surface we shall equate them.

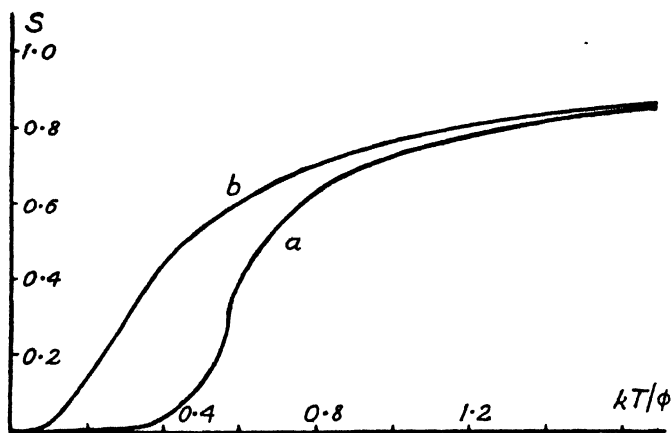


FIG. 7.

The problem is solved by studying the effect on the partition function for the surface by adding an extra row of molecules. The final result is, for the  $(100)$  surface

$$s = 1 - \frac{1}{2} (1 + 2k_2 K_1/\pi) \coth H, \quad (3)$$

where

$$H = \phi_1/2kT; \quad k_2 = 2 \tanh^2 H - 1;$$

$$K_1 = K(k_1) = \int_0^{\pi/2} \frac{d\omega}{(1 - k_1^2 \sin^2 \omega)^{1/2}}; \quad k_1^2 + k_2^2 = 1 \quad (4)$$

$K_1$  is the complete elliptic integral of the first kind. A graph of  $s$  against  $T$  is shown in Fig. 7 (a). The curve possesses a vertical tangent at  $T = T_c$  given by  $k_1 = 1$  or  $k_2 = 0$ , i.e., by  $\sinh H_c = 1$ ,

$$\text{or} \quad H_c = \phi_1/2kT_c = \ln \cot \frac{\pi}{8} \sim 0.9 \quad (5)$$

If we had assumed the jumps to be independent, the result would have been

$$s = \frac{2 \exp(-\phi_1/2kT)}{1 + \exp(-\phi_1/2kT)}, \quad (6)$$

which gives rise to curve  $b$  in Fig. 7.

In the absence of square symmetry, Onsager<sup>6,7</sup> has shown that that preceding condition becomes

$$\sinh H_c \sinh H_c' = 1, (H_c' = \varphi_2/2kT_c), \quad (7)$$

so that we can apply our result to the (110) surface of our model. In the case of this surface we have a rectangular lattice with energy of interaction  $\varphi_1/2$  in one direction and  $\varphi_2/2$  in the other, where  $\varphi_2$  is the strength of the second nearest neighbour interaction. Consequently, if the second nearest neighbour interactions are small enough, the critical temperature can be as low as we like.

The value of  $T_c$  has been worked out for all surfaces in the symmetrical case.<sup>8</sup> The result is

$$\text{gd } H_c = \pi/Z, \quad (8)$$

where  $\text{gd } x$  is the Gudermannian function, and  $Z$  is the number of nearest neighbours in the surface, of a given molecule in the surface, when it is as flat as possible. There are three cases: (a) surface lattice triangular,  $Z = 6$ ; (b) surface lattice square,  $Z = 4$ ; (c) surface lattice hexagonal,  $Z = 3$ . For these cases we have respectively from (8),

$$\exp(2H_c) = 3, \quad (9)$$

$$\sinh H_c = 1, \quad (10)$$

$$\cosh H_c = 2. \quad (11)$$

Eqn. (9) enables us to give the critical temperature for the (111) face of a simple cubic crystal. The surface in this case has a triangular lattice and the nearest neighbour interactions in the surface are due to the second nearest neighbours in the crystal lattice. From (9) we get

$$2H_c' = \varphi_2/kT_c \sim 1.1.$$

In Table I we give  $T_c$  under the assumption that  $\varphi_1 = 0.2$  eV,  $\varphi_1/\varphi_2 = 8$ . If  $T$  is much below  $T_c$ , the concentration of jumps is almost entirely due to the presence of adsorbed molecules.

We see that the critical temperature gives the boundary separating the régime where the jump concentration is negligible and surface nucleation is required for growth,  $T < T_c$ , from that régime  $T > T_c$ , where the jump concentration is high, and no nucleation is required for growth. If  $T > T_c$  the surface

will grow rapidly and disappear leaving only the slow growing faces for which  $T < T_c$ . At ordinary temperatures then, the transition phenomena will only occur for non-habit faces.

Broadly speaking, we have shown that close-packed surfaces will not grow at low supersaturations (see Part II) because of the need for surface nucleation. However, we have assumed that the crystal lattice is perfect. It is clear that the structure of a close-packed surface at equilibrium is extremely sensitive to lattice imperfections, and as Frank<sup>9</sup> has pointed out the presence of a few dislocations terminating in the surface ensures the presence of suitable sites for condensation or evaporation. In fact, dislocations again produce a stepped surface. The perfect close-packed surface will rarely, if ever, occur in reality.

*H. H. Wills Physical Laboratory,  
University of Bristol.*

\* Burton, Cabrera and Frank, *Nature*, 1949, 163, 398. Frank, This Discussion.

TABLE I

Lattice	Surface	$T_c$
Simple cubic . . . . .	(100)	1000° C
	(110)	400° C
	(111)	-30° C
Face-centred cubic . . . . .	(111)	1700° C

# CRYSTAL GROWTH AND SURFACE STRUCTURE

## Part II

BY N. CABRERA AND W. K. BURTON \*

**1. Introduction.** In Part I of this paper we studied the microscopic equilibrium structure of crystal surfaces and distinguished two kinds, stepped surfaces and close-packed or saturated surfaces. This second type is essentially the same as the first one, when the temperature is above a certain critical value. Below this temperature the close-packed surfaces behave in quite a different way.

In Part II we treat the kinetic problem of the growth of these surfaces from the vapour; in all cases it is necessary to consider the diffusion of adsorbed atoms on the surface of the crystal. First, we treat the growth of stepped surfaces; quantitative formulæ are given as a function of the inclination. The growth of close-packed surfaces below their critical temperature by the mechanism of two-dimensional nucleation is also considered. Treatments of two-dimensional nucleation have been given by several authors, especially by Becker and Döring,<sup>1</sup> neglecting the effect of the diffusion of adsorbed atoms. We discuss in this paper the influence of this diffusion and we show that in spite of the fact that the predicted rate of growth is perhaps different from that given by Becker and Döring, it will certainly not account for the experimental fact that in the small number of cases where a critical supersaturation for growth has been observed, it is of the order 0.01 at most. Finally, a treatment of the initiation of growth of an imperfect crystal containing a random distribution of dislocations is given, and is shown to be in agreement with experiment. The results of this work will be given here in a preliminary way; the complete treatment will be published elsewhere.

**2. Growth of Stepped Surfaces.** In Part I we showed that each step in the surface contains in equilibrium a very high concentration of Frenkel kinks, much higher than the concentration of adsorbed atoms in the edge of the step. The equilibrium concentration of adsorbed atoms on the surface is

$$\frac{1}{a^2} e^{-W_s/kT}, \quad . \quad . \quad . \quad . \quad . \quad (1)$$

where  $a$  is the interatomic distance and  $W_s$  the evaporation energy from the step into the surface.

When the vapour pressure is increased by a factor  $\alpha$  (saturation ratio =  $\alpha$ ; supersaturation =  $\alpha - 1$ ), the concentration of adsorbed atoms remains practically equal to that in equilibrium near the steps, because of the high concentration of Frenkel kinks in them. The saturation ratio  $\alpha_s$  on the surface increases from the value 1 near the steps to a maximum value between the steps. The current of atoms condensing into the steps will be controlled essentially by the diffusion of adsorbed atoms on the surface.

<sup>1</sup> Becker and Döring, *Ann. Physik*, 1935, **24**, 719.

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The actual variation of  $\alpha_s$  can be obtained solving the corresponding diffusion problem, the result being

$$\alpha - \alpha_s(x) = (\alpha - 1) \frac{\cosh \beta x}{\cosh \beta x_0}, \quad (2)$$

where  $xa$  is the distance counted from half-way between the steps,  $2x_0a$  is the mean distance between them, and

$$\beta^2 = \exp \{ - (W_1 - U)/kT \}, \quad (3)$$

$W_1$  being the energy necessary to take an adsorbed atom from the surface into the vapour ( $W = W_s + W_1$  is the total evaporation energy) and  $U$  the activation energy for diffusion on the surface. Therefore  $\beta^2$  is the ratio between the probabilities for an adsorbed atom to evaporate into the vapour and to diffuse on the surface. Usually  $W_1 > U$ ; if  $U$  is very small,  $\beta$  will also be very small, and the concentration of adsorbed atoms on the surface will be practically uniform and equal to that in equilibrium, unless  $x_0$  is very big. As  $U$  increases the non-uniformity on the surface becomes more important. If  $W_1 < U$ , the diffusion on the surface does not play any role, the condensation into the steps takes place directly from the vapour. We do not think that this is likely to occur.

In order to illustrate the values that  $\beta$  can take, let us consider a face-centred cubic crystal with a stepped surface consisting of terraces (1, 1, 1) and steps in any direction, and let us call  $\phi$  the interaction energy between nearest neighbours. Then  $W_1 \sim 3\phi$  and  $U \sim \phi$ , therefore

$$\beta = \exp \{ - \phi/kT \} \sim 0.05,$$

if, e.g.,  $\phi/kT \sim 3$ .

The current  $j$  of atoms condensing per sec. per cm. into each step will be equal to the current of atoms condensing from the vapour on the surface between two steps; therefore, from (2),

$$= 2 \frac{\nu}{a} e^{-W/kT} \frac{1}{\beta} \tanh \beta x_0, \quad (4)$$

where  $\nu$  is the frequency of vibration of the adsorbed atoms. The velocity of advance of the steps,

$$v = a^2 j,$$

will be a function of the distance between steps. For  $x_0\beta \ll 1$  the velocity of the steps is proportional to the distance between them; as  $x_0$  increases above  $\beta^{-1}$ ,  $v$  tends to a maximum value  $v_\infty$  given by

$$v_\infty = 2(\alpha - 1)\nu a \exp \{ - (W + W_s + U)/2kT \}, \quad (5)$$

which represents the velocity with which a *single* step would move. For the example considered above

$$\exp \{ - (W + W_s + U)/kT \} = \exp \{ - 5\phi/kT \};$$

putting  $\nu \sim 10^{12}$  sec.<sup>-1</sup>,  $a \sim 10^{-8}$  cm.,  $\phi/kT \sim 3$  and  $\alpha - 1 \sim 10^{-2}$ , we get  $v_\infty \sim 10^{-5}$  cm./sec.

The total current  $J$  of atoms adsorbed per cm.<sup>2</sup> per sec. is

$$J = Nj,$$

where  $N = 1/2x_0a$  is the number of steps per cm., therefore

$$Na \geq \frac{1}{2}\beta; \quad J_0 = (\alpha - 1) \frac{\nu}{a^2} e^{-W/kT} \quad (6)$$

$$Na \leq \frac{1}{2}\beta; \quad J = 2NaJ_0/\beta \quad (7)$$

For values of  $Na \sim \beta$ , the current  $J$  will depend on the distribution of steps in the surface. The curve, Fig. 1, has been calculated assuming a random

distribution. For  $Na \sim 0$  and  $Na \sim 1$  the stepped surface transforms into close-packed surfaces of quite different character (see § 3). We see that the rate of growth of stepped surfaces is practically given by formula (6), down to small values of  $Na$ .

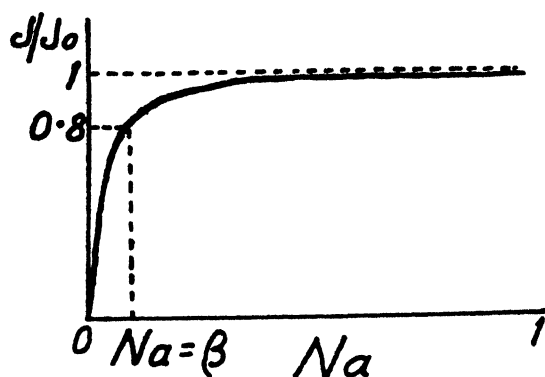


FIG. 1.—Rate of growth of stepped surfaces as a function of the number  $N$  of steps per cm.

Recent experimental work by Graf,<sup>2</sup> and Mahl and Stranski,<sup>3</sup> suggests that the stepped surfaces present a striated structure during growth from the vapour, the distance between the striations being of the order of  $10^{-4}$  cm. We showed in Part I that this striated structure cannot be in equilibrium with the vapour, the surfaces always having a tendency to be as flat as possible. We believe that the formation of these striations has an essentially kinetical character and is probably related to the fact that the velocity of displacement of the steps is a function of the distance between its nearest neighbours. A correct theory has not yet been obtained.

**3. Growth of Close-packed Surfaces.** Let us consider now the surfaces without steps. The surfaces with small indices will correspond to this type. We considered in Part I the equilibrium structure of these surfaces, and we have shown that they will remain practically flat if the temperature is below a certain critical temperature, but they will contain a considerable number of Frenkel kinks if the temperature is above it. It is easy to show that for close-packed surfaces above their critical temperature the rate of growth is again given by (6). This type of surface can occur, for instance, as the limiting case of a stepped surface when  $Na \sim 1$ .

**SURFACE NUCLEATION PROCESS.**—Let us now consider the growth of habit surfaces, where Frenkel kinks do not occur. A long time ago Gibbs, and later on Volmer, suggested that the growth of these surfaces requires a two-dimensional nucleation process.

The theory of nucleation, especially in the case of formation of three-dimensional liquid or solid nuclei from the vapour, has been developed by Volmer,<sup>4</sup> Stranski<sup>5</sup> and Becker and Döring.<sup>1</sup> In that case the diffusion in the vapour plays a small role and the supersaturation is practically the same all over the volume. The tendency for small nuclei to evaporate is very big and the supersaturation required for them to grow has to be high

<sup>2</sup> Graf, *Z. Elektrochem.*, 1942, **48**, 181.

<sup>3</sup> Mahl and Stranski, *Z. Metallkunde*, 1943, **35**, 147.

<sup>4</sup> Volmer, *Z. physik. Chem.*, 1926, **119**, 277.

<sup>5</sup> Kaishew and Stranski, *Z. physik. Chem. B*, 1934, **26**, 317.

( $\alpha - 1$  of the order of 4). The number of nuclei of critical size formed per sec. per cm.<sup>3</sup> turns out to be of the form,

$$I = B e^{-A/kT}, \quad (8)$$

where  $B \sim 10^{40}$  cm.<sup>-3</sup> sec.<sup>-1</sup> and  $A$  is the increase in free energy necessary for the formation of a nucleus of critical size;  $A$  is tremendously high for all values of  $\alpha$  smaller than 4.

Frenkel<sup>6</sup> and Becker<sup>7</sup> applied the same ideas to cases in which there is a diffusion of the atoms condensing into the nuclei, such as occurs in precipitation in a supersaturated alloy. They assumed that the only change in formula (8) to be made in this case is to multiply by a factor  $\exp(-U/kT)$ , where  $U$  is the activation energy for diffusion. Actually this assumption is not entirely correct; it implies that the supersaturation is the same all over the volume, which is not true if diffusion exists.

The surface nucleation required for the growth of habit surfaces can be treated in a similar way, and the diffusion of atoms on the surface has also to be taken into account.

Before studying two-dimensional nucleation, let us consider the growth of a single nucleus on the top of a habit face. We assume a circular shape of radius  $\rho a$ ; if  $\mu$  is the number of atoms contained in it,

$$\rho = \sqrt{\mu/\pi}.$$

If the total energy of a nucleus of  $\mu = \pi\rho^2$  atoms is

$$\pi\rho^2 W_s - 2\pi\rho\gamma,$$

where  $\gamma$  is the edge energy per atom, the mean evaporation energy from the nucleus is

$$W(\rho) = W_s - (\gamma/\rho) \quad (9)$$

Let  $\alpha$  be the supersaturation ratio in the vapour; assuming the nucleus to be big enough, it will not change appreciably before a steady distribution of adsorbed atoms around it has been formed. Under these conditions the diffusion problem can be solved, and the supersaturation ratio  $\alpha_s(r)$  on the surface around the nucleus is given by

$$\begin{aligned} r < \rho, \quad \alpha - \alpha_s(r) &= [\alpha - \alpha_s(\rho)] \frac{I_0(\beta r)}{I_0(\beta \rho)}, \\ r > \rho, \quad \alpha - \alpha_s(r) &= [\alpha - \alpha_s(\rho)] \frac{K_0(\beta r)}{K_0(\beta \rho)}. \end{aligned} \quad (10)$$

$I_0$  and  $K_0$  are the Bessel functions of first and second kind with imaginary argument.  $\alpha_s(\rho)$  is the supersaturation ratio near the edge, which by definition is

$$\alpha_s(\rho) = \alpha_s^e(\rho) + \frac{j(\rho)}{4\pi\rho v} e^{(W_s+U)/kT},$$

where

$$\alpha_s^e(\rho) = \exp\{\gamma/\rho kT\}$$

is the supersaturation ratio which would be in equilibrium with the nucleus, and  $j(\rho)$  is the current of atoms condensing per sec. into the nucleus. The current is then calculated as the number of atoms condensing from the vapour per sec. all over the surface. The result is

$$j(\rho) = 4\pi\rho v [\alpha - \alpha_s^e(\rho)] \frac{e^{-W/kT}}{\beta^2 [2\rho \cdot I_0(\beta \rho) \cdot K_0(\beta \rho) + 1]} \quad (11)$$

<sup>6</sup> Frenkel, *Soujet Physik*, 1932, 1, 498.

<sup>7</sup> Becker, *Ann. Physik*, 1938, 32, 128; *Proc. Physic. Soc.*, 1940, 52, 71.



The radial velocity is

$$v(\rho) = aj(\rho)/2\pi\rho.$$

Current and velocity change sign when  $\alpha = \alpha_s^c(\rho)$ , which defines the critical nucleus to have a radius  $\rho_c$  given by

$$\rho_c = \gamma/kT \ln \alpha. \quad (12)$$

For  $\rho < \rho_c$  the nucleus evaporates, for  $\rho > \rho_c$  the nucleus grows. The maximum velocity of growth of the nucleus, for  $\rho \gg \rho_c$  and  $\beta\rho \gg 1$ , reduces of course to formula (5). If  $\rho_c$  is big, such that  $\beta\rho_c > 1$ , the velocity is given by

$$v(\rho) = v_\infty \left(1 - \frac{\rho_c}{\rho}\right), \quad (13)$$

valid for  $\rho \gg \rho_c$ . For  $\beta\rho_c < 1$ , the velocity curve becomes steeper near the critical size. This is illustrated in Fig. 2. The concentration  $\alpha_s(\rho)$  near the edge of the nucleus is seen to be practically the same as  $\alpha_s^c(\rho)$ ; it is a little bigger when  $\rho < \rho_c$  and smaller when  $\rho > \rho_c$ . The correction increases as  $\rho$  decreases but remains small.

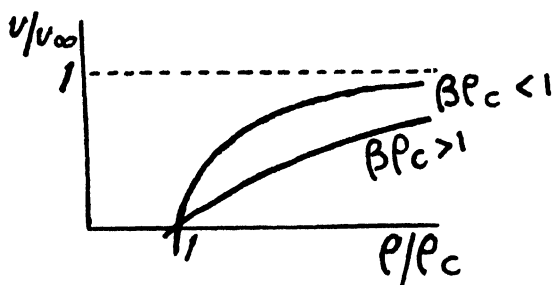


FIG. 2.—Radial velocity of a nucleus bigger than the critical size.

Now let us consider the current  $J$  of atoms condensing per  $\text{cm}^2$  per sec. on a close-packed surface. In a steady state, when a constant supersaturation  $\alpha - 1$  is maintained in the vapour, there will be on the surface a stationary distribution  $n_\mu$  of nuclei of different sizes. They grow until they collide with other nuclei in the same atomic layer and disappear; we can take this into account by assuming that there is a certain maximum value  $M$  for  $\mu$ . Given the actual distribution  $n_\mu$ , there will be a surface supersaturation ratio  $\alpha_s(x, y)$ , a function of position. The total current  $J$  will be equal to

$$J = (\alpha - \bar{\alpha}_s) \frac{v}{a^2} e^{-W/kT} \quad (14)$$

where  $\bar{\alpha}_s$  is the mean value of  $\alpha_s$  all over the surface. When  $\alpha$  is very near 1, the number of nuclei on the surface is small, and therefore most of the surface has a supersaturation ratio  $\alpha_s \sim \alpha$ ; consequently  $\bar{\alpha}_s \sim \alpha$  and the current  $J$  will be very small. As  $\alpha$  increases the number of nuclei increases and the mean value  $\alpha_s$  decreases, becoming  $\bar{\alpha}_s \sim 1$  when the proportion of big nuclei is high and the distances between them small. We expect therefore that  $J$  as a function of  $\alpha$  will be represented by a curve such as is illustrated in Fig. 3, where the straight line corresponds to the formula (6).

The calculation of  $\bar{\alpha}_s$  as a function of  $\alpha$  is a very difficult problem; it requires, of course, the knowledge of the distribution  $n_\mu$  of nuclei of different size as a function of  $\alpha$ . Nevertheless for small values of  $\alpha - 1$  for which the current  $J$  remains small (region OA in Fig. 3), it can be estimated using a method proposed by Becker and Döring<sup>1</sup> which we cannot develop here.

We assume that the growth of any nucleus is due fundamentally to the condensation of single adsorbed atoms; this assumption is correct only for a small density of nuclei, and therefore when  $\alpha - 1$  is small. Under these conditions, the number  $I$  of nuclei of any size  $\mu$  formed per sec. per cm.<sup>2</sup> from nuclei  $\mu - 1$  can be calculated if the ratios  $q(\mu)/a(\mu)$  of the mean probability of evaporation  $q(\mu)$  to that of growth  $a(\mu)$  for every nucleus are known. The current  $J$  of atoms condensing per sec. per cm.<sup>2</sup> is then calculated from  $J = IM$ , where  $M$  is the maximum value of  $\mu$ . In general,

$$q(\mu)/a(\mu) = \alpha_s'(\mu)/\alpha_s(\mu),$$

the ratio of the supersaturation ratio which would be in equilibrium with the nucleus to that actually existing near it.

Let us first assume that the influence of the diffusion on the surface is unimportant, and therefore  $\alpha_s(\mu) = \alpha$  for all nuclei. Under these conditions one gets for the current  $J$  the expression,

$$J = \frac{\gamma}{a^2} e^{-(W+U)/kT} e^{-A_c/3kT}, \quad (15)$$

where

$$A_c = -\pi\rho_c^2 kT \ln \alpha + 2\pi\rho_c \gamma = \gamma^2 \pi / kT \ln \alpha,$$

is the increase in free energy necessary for the formation of a critical nucleus. The factor  $\frac{1}{3}$  multiplying  $A_c$  comes from the calculation of  $M$ , which happens to contain a factor  $\exp\{\frac{1}{3}A_c/kT\}$ ; this of course is assuming that the surface itself is much bigger than  $M$ . It is easy to see that expression (15) will give a negligible rate of growth, unless  $\alpha$  is of the order of 2. Actually, according to Volmer and Schultze<sup>8</sup> a linear rate of growth is observed above  $\alpha - 1 \sim 10^{-2}$ . For this supersaturation, taking

$$\gamma \sim \phi, \text{ and } \phi/kT \sim 3,$$

the exponent in (15) becomes  $A/3kT \sim 10^3$ , and therefore no growth should occur at all.

Let us now consider the influence of diffusion. This is a very difficult problem, for which only a qualitative answer has been found.

There are two conflicting effects. First of all, the nuclei bigger than the critical size, which are therefore on the average growing, decrease the concentration of adsorbed atoms in the neighbourhood of their edge, with the result that the current of condensation in these nuclei is now much lower than before. On the other hand, the nuclei smaller than the critical size, which are in the average evaporating, may tend to increase the concentration of adsorbed atoms around their edge, and consequently the probability for them to grow further is higher than it was before. From the study of the evaporation of a *single* nucleus we showed that there was an increase of concentration near its edge. Nevertheless, we do not think that the same considerations apply to the assembly of nuclei in the nucleation process. In that case owing to the fact that the distribution of nuclei  $n_\mu$  is a decreasing function of  $\mu$  more nuclei are coming to the size  $\mu$  per sec. by growth from  $\mu - 1$  than nuclei coming by evaporation from  $\mu + 1$ , and this difference increases when  $\mu$  decreases; therefore we think that the supersaturation ratio  $\alpha_s(\mu)$  near the nuclei  $\mu$  must tend to the value  $\alpha$  for values of  $\mu$  not very small compared with the critical size  $\mu_c$ .

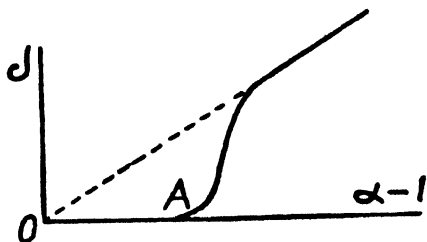


FIG. 3.—Rate of growth  $J$  as a function of  $\alpha - 1$ .

<sup>8</sup> Volmer and Schultze, *Z. physik. Chem. A*, 1931, 156, 1.

As an illustration, let us suppose that

$$\alpha_s(\mu) = \alpha$$

for nuclei  $\mu$  smaller than a certain size  $\mu_0$ , and

$$\alpha_s(\mu) = \alpha_s'(\mu)$$

for bigger nuclei. Then the total current  $J$  can be shown to be

$$J = \frac{\nu}{a^2} e^{-(W+U)/kT} e^{-A_0/kT} \quad (16)$$

where

$$A_0 = -\pi\rho_0^2 kT \ln \alpha + 2\pi\rho_0\gamma,$$

is the increase in free energy necessary for the formation of a nucleus of size  $\mu_0 = \pi\rho_0^2$ . This formula will give a bigger current than (15) if  $A_0 < A_c/3$ , therefore if  $\mu_0 < 0.04 \times \mu_c$ . This is reasonable if we consider that for supersaturations of the order  $\alpha - 1 \sim 10^{-2}$ , assuming always

$$\gamma/kT \sim \phi/kT \sim 3,$$

$\mu_c$  is of the order  $10^5$ . On the other hand, it can be shown that formula (16) will account for the observed rate of growth at supersaturations of the order  $10^{-2}$ , only if  $\mu_0 < 10$  which is certainly too small. We conclude from these considerations that, in spite of the fact that the diffusion perhaps changes the current given by the simple nucleation theory, it does *not* agree with the current experimentally observed.

**ROLE OF DISLOCATIONS IN CRYSTAL GROWTH.**—According to Frank,<sup>9</sup> the surface of any real crystal must contain a certain number of dislocations, with a screw component, terminating in the surface and producing steps which do *not* disappear during growth. Under these conditions the two-dimensional nucleation is no longer necessary. The current of condensation in the special case of a random distribution of these dislocations can be estimated in the following way.

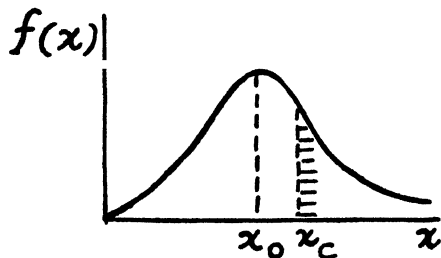


FIG. 4.—Distributions of steps of different length  $x$  between neighbour dislocations of different sign.

between  $x$  and  $x + dx$  (times the interatomic distance  $a$ ) will be represented by a function illustrated in Fig. 4. If we assume that the steps are always formed between two nearest neighbour dislocations of different sign, then

$$f(x) = 2 \frac{x}{x_0^2} e^{-\left(\frac{x}{x_0}\right)^2}, \quad (17)$$

where  $x_0^2 = \frac{2}{\pi} \frac{1}{N a^2}$ .

Let  $\alpha - 1$  be the supersaturation in the vapour. Then all the steps of length  $x$  bigger than the diameter  $x_c$  of the critical nucleus, given by

$$x_c = \frac{2\gamma}{kT \ln \alpha} \sim \frac{2\gamma}{kT(\alpha - 1)}, \quad (18)$$

<sup>9</sup> Frank, This Discussion.

will grow freely, until they collide with other steps. The length of the steps remains always of the order of  $x$ . The steps of length  $x < x_c$  will have a very small probability for growth; we shall neglect their contribution to the total current.

The current of atoms condensing per sec. into the steps of length  $x$ ,  $j(x)$  will also be a function of the distance to the nearest neighbour steps; nevertheless, provided the condition  $\beta x_0 \gg 1$  is satisfied (see §1), we can use formula (5), that is to say,

$$j(x) = \frac{2\nu}{\beta} e^{-W/kT} (\alpha - 1)x.$$

The total current  $J$  of atoms condensing per sec. per cm.<sup>2</sup> in surface will now be given by

$$J = \frac{N}{2} \int_{x_c}^{\infty} j(x)f(x)dx. \quad (19)$$

Fig. 5 illustrates the current obtained from (19) as a function of  $(\alpha - 1)$  and for a given value of  $N$ . For  $x_c > x_0$  ( $\alpha - 1$  very small),  $J$  is given by the expression

$$J = \frac{Na^2\nu}{\beta a^2} e^{-W/kT} \exp \left\{ - \left( \frac{x_c}{x_0} \right)^2 \right\},$$

where  $x_0$  and  $x_c$  are given by (17) and (18). For  $x_c < x_0$  the current tends to a linear law of the form

$$J = \frac{N^{1/2} a \nu}{\beta a^2} e^{-W/kT} (\alpha - 1). \quad (20)$$

The critical supersaturation (point C in Fig. 5) for which the current becomes practically linear is given by  $x_0/x_c \sim 2$ ,

$$\alpha - 1 \sim 2\sqrt{2\pi} \frac{\gamma}{kT} Na^{1/2}. \quad (21)$$

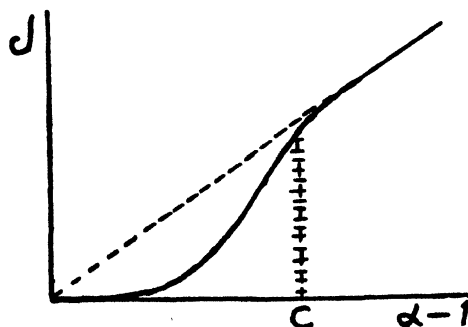


FIG. 5.—Rate of growth of a close-packed surface containing  $N$  dislocations per cm.<sup>2</sup> as a function of the supersaturation  $\alpha - 1$ .

The general shape of the curve represented in Fig. 5 agrees with the experimental results of Volmer and Schultze,<sup>8</sup> for the growth of iodine crystals at 0° C. The critical supersaturation is in their case  $\alpha - 1 \sim 10^{-2}$ , which agrees with the value given by (21), taking  $\gamma/kT \sim 3$  and assuming  $N = 10^8$  cm.<sup>-2</sup> which agrees with the value generally assumed to explain the mechanical properties of crystals.

Volmer and Schultze (loc. cit.) observe also a linear law of growth, as a function of  $\alpha - 1$ , for  $\alpha - 1 > 10^{-2}$ . The experimental value of the rate of growth for  $\alpha - 1 = 10^{-2}$  is of the order of  $10^2$  atomic layers per sec.

Formula (20) is strictly speaking only applicable to simple monoatomic substances; in order to apply it to complicated structures as iodine, we have just to calculate  $W$  and  $\nu$  in such a way to account for the saturation vapour pressure of iodine. Using the experimental values of Gillespie and Fraser,<sup>10</sup> one obtains  $W = 0.7$  eV,  $\nu = 0.4 \times 10^{17}$  sec.<sup>-1</sup>.

Putting these values, and  $N \sim 10^8$  cm.<sup>-2</sup>,  $\beta \sim 10^{-2}$ , into formula (20) one obtains a rate of growth of the order of the experimental value.

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<sup>10</sup> Gillespie and Fraser, *J. Amer. Chem. Soc.*, 1936, **58**, 2260.

## THE INFLUENCE OF DISLOCATIONS ON CRYSTAL GROWTH

BY F. C. FRANK

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The kinetic theory of the nucleation of new phases, developed especially by Volmer,<sup>1</sup> by Farkas,<sup>2</sup> by Kaischew and Stranski<sup>3</sup> and by Becker and Döring,<sup>4</sup> indicates that under typical conditions \* the self-nucleation from vapour of new crystals, new liquid drops and fresh two-dimensional monolayers of molecules on a "saturated" crystal face require respectively supersaturations of the vapour by factors of typically 10, 5 and 1.5 respectively in order to proceed at appreciable rates. Experimentally, the first two of these figures are apparently correct: but the third is much larger than the actual supersaturation required to cause further growth of a crystal already formed. In fact, the existence of a critical finite supersaturation for further growth has only been established for a few materials, and then for individual faces of individual crystals, being different from case to case; at the most it is about 1 %. Volmer and Schultze,<sup>5</sup> who found a critical supersaturation of 0.8 % for the growth of an iodine crystal from the vapour, interpreted this as the critical supersaturation for two-dimensional nucleation: but the quantitative discrepancy is far too great (for details of the growth rate formulæ, see the contributions of Burton and Cabrera to this Discussion).

However, this discrepancy is not in the least surprising. One ought not to expect that any visible crystal will exhibit a completed perfect face needing fresh two-dimensional nucleation in order to grow. Investigation of the mechanical properties of solids shows that no macroscopic specimen ever exhibits the theoretical strength of the perfect crystal; and this enormous discrepancy (a factor of 100, say, and *more* for "good"

<sup>1</sup> Volmer and Weber, *Z. physik. Chem.*, 1926, **119**, 277. Volmer, *Kinetik der Phasenbildung* (Leipzig, 1939).

<sup>2</sup> Farkas, *Z. physik. Chem.*, 1927, **125**, 236.

<sup>3</sup> Kaischew and Stranski, *Z. physik. Chem. B*, 1934, **26**, 317; *Physik. Z.*, 1935, **36**, 393.

<sup>4</sup> Becker and Döring, *Ann. Physik*, 1935, **24**, 719.

<sup>5</sup> Volmer and Schultze, *Z. physik. Chem. A*, 1931, **156**, 1.

\* Typical conditions may be taken as such that the vapour pressure lies within a few powers of 10 of 1 mm. Hg: or the temperature between about 0.5 and 0.8 times  $T_b$ , the boiling point in °K.

crystals) is attributed to the presence of dislocations. In the early stages of the development of dislocation theory by Polanyi,<sup>6</sup> Orowan<sup>7</sup> and Taylor,<sup>8</sup> only one aspect of the dislocation was recognized, in which the displacement direction was normal to the dislocation line. In 1939 Burgers<sup>9</sup> drew attention to the "screw" form assumed by the dislocation when the displacement is parallel to the dislocation line, and the developments of dislocation theory by Mott and Nabarro,<sup>10</sup> Frank<sup>11</sup> and others emphasize the fact that dislocation lines can be curved and exhibit any orientation. Fig. 1 shows (a) as a continuous deformation of a plane, (b) in a block model, the form of a simple cubic crystal when a screw dislocation emerges normally at the

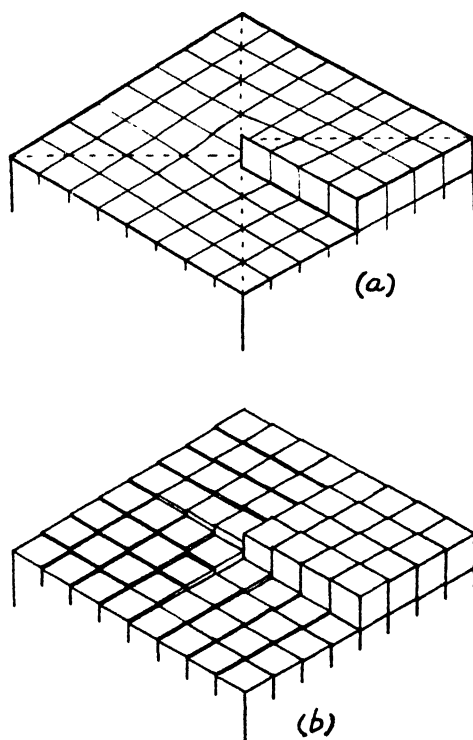


FIG. 1.—The end of a screw dislocation.

cube-face. It is clear that when dislocations of this type are present, the crystal face always has exposed molecular terraces on which growth can continue, and the need for fresh two-dimensional nucleation never arises. If just one dislocation of this type emerges at the centre of the face, that crystal face can grow perpetually "up a spiral staircase." If there are two, respectively right- and left-handed, we can show that the terrace connecting them will grow indefinitely if the supersaturation is raised to such a value that the diameter,  $l_0$ , of the critical two-dimensional nucleus is less than the distance between them. More precisely, since the critical nucleus is not

<sup>6</sup> Polanyi, *Z. Physik*, 1934, **89**, 660.

<sup>7</sup> Orowan, *Z. Physik*, 1934, **89**, 634.

<sup>8</sup> Taylor, *Proc. Roy. Soc. A*, 1934, **145**, 362.

<sup>9</sup> Burgers, *Proc. Kon. Ned. Akad. Wet.*, 1939, **42**, 293.

<sup>10</sup> Mott and Nabarro, *The Strength of Solids* (Physical Society, London), 1948, p. 1.

<sup>11</sup> Frank, *ibid.*, p. 46.

circular, we should say that growth occurs when the critical nucleus, correctly oriented, will pass between two points in the positions of the two dislocations. Likewise when a single dislocation is close to the boundary of the face, growth occurs if the critical nucleus will pass between the dislocation and the boundary. If it will not pass, the terrace rests in a position corresponding to a portion of the boundary of the critical nucleus (Fig. 2). For rough calculations we may approximate the boundary as a circle of radius  $\frac{1}{2}l_0$ , and we have a close analogy to the equilibrium (or non-equilibrium) of a bubble at an orifice.

Measuring in molecular spacings, we have

$$l_0 = \phi/kT \ln \alpha,$$

where  $\alpha$  is the saturation ratio: since the supersaturation is small,  $(\alpha - 1)$  may be written in place of  $\ln \alpha$ .  $\phi$  is the neighbour-neighbour binding energy of the crystal. For a rule of thumb we may use Trouton's rule and take  $\phi/kT$  as  $3.5 T_b/T$ . Thus at an absolute temperature of 0.6 of the boiling point of the material, and a supersaturation of 1%,  $l_0$  is about 600 molecular spacings: or 6000 at 0.1%.

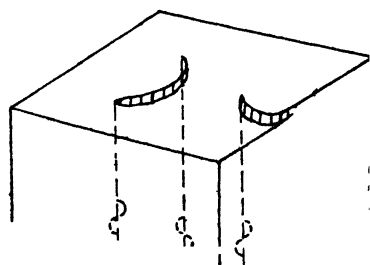


FIG. 2.—One right-handed and two left-handed screw dislocations ending in a crystal face.

In the theory of mechanical deformation, it is commonly estimated that in an annealed metal crystal there are about  $10^8$  dislocation lines intersecting each square centimetre. This appears to be the limiting perfection attainable in metals, and would be classed, indeed, as high perfection from the point of view of X-ray diffraction. (This limit may well be connected with the impurity content.) This is only an order-of-magnitude estimate, and does not necessarily apply to other materials than metals: but it serves as a

guide, and suggests that critical supersaturations of the order of a fraction of 1% are reasonably to be expected.

We are perforce limited for quantitative discussion to the case of growth from the vapour of homopolar crystals, since the classical nucleation rate theory has not been quantitatively developed for any other case. But experimental indications, and such theoretical guesswork as we can make, suggest that the conditions governing the growth from solution of crystals, including ionic crystals, are substantially similar. It is possible, but less certain, that the same is true of growth from the melt. Perhaps the most distinctively different case is that of the growth, either from vapour or solution, of crystals of highly non-equiaxed organic molecules. There are indications that in such cases growth proceeds through the formation of adsorption films, dense, but differing in molecular orientation from the bulk crystal (e.g., liquid, or liquid-crystalline) within which subsequent rearrangement occurs.

The general importance of dislocations for crystal growth accounts immediately for many observations, such as the individual behaviour of each crystal face, particularly on the microscopic scale, leading sometimes to such unexpected results as the formation of lath-shaped crystals for a lattice of cubic symmetry.

Under steady uniform supersaturation the terrace attached to an isolated growth-promoting dislocation in a crystal otherwise perfect in its neighbourhood will grow outwards in a spiral of which the spacing between turns, and the rate of their advance, will be uniform at a considerable distance from

the centre. Near the centre the rate of advance must be less, since the radius of curvature of the spiral terrace-line must remain less than  $\frac{1}{2}l_0$ . At given supersaturation and distance from other terrace-lines, the rate of advance,  $v$ , is an increasing function of the radius of curvature, being 0 when it is  $\frac{1}{2}l_0$  and, say,  $v_\infty$  when the terrace becomes straight. Supposing it increased very steeply to  $v_\infty$  for a very small increase of the radius of curvature above  $\frac{1}{2}l_0$ , the inner portion of the spiral would be an arc of a circle of this radius, and the spiral would make  $v_\infty/\pi l_0$  turns per second. The spacing of turns in the outer region of the spiral would then be  $\pi l_0$ . Actually  $v$  will not increase infinitely steeply to  $v_\infty$ , so that the number of turns per second will be less, and the spacing greater, but still of the same order of magnitude: let us guess  $2\pi l_0$ . Macroscopically this spirally terraced hill would appear to be a flat cone, with its sides inclined at an angle of  $\frac{1}{2}\pi l_0$  radians to the true lattice surface—say, 1 min. for a supersaturation of 1 %, and in other cases pro-

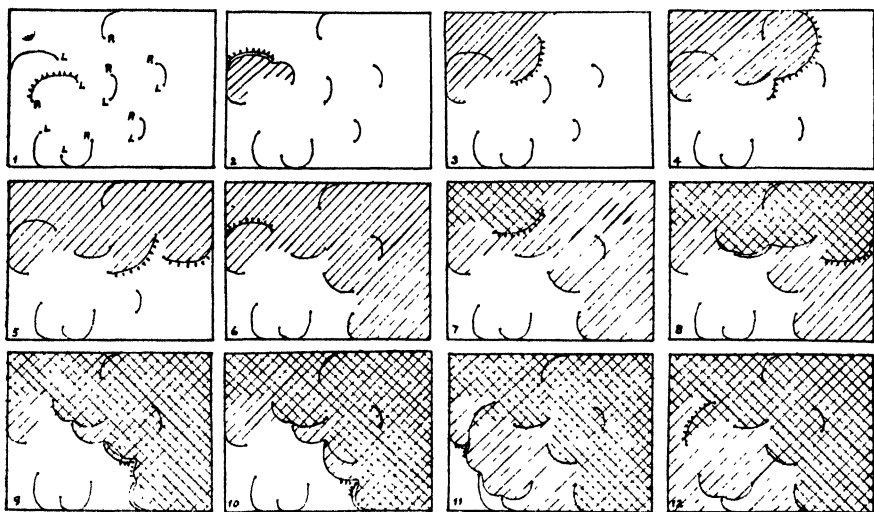


FIG. 3.—The influence of random dislocations on crystal growth.

portional to the supersaturation. We have so far neglected the dependence of the rate of advance of the terrace on its direction. On this account, instead of a flat cone, we shall have a more or less sharply defined flat pyramid having the symmetry appropriate to the crystal face.

When growth spreads from an isolated pair of dislocations, respectively right- and left-handed, the observable result will be practically the same provided that the supersaturation suffices to make  $l_0$  less than their separation. If it is not, no growth occurs at all. If it is, the terrace-line connecting them repeatedly spreads out on one side, wraps round, and meets itself on the other side: thus forming a short connecting terrace-line again, and an outward-growing closed loop. The rate of formation of such loops will be the same in order of magnitude as the rate of formation of turns of the spiral from a single dislocation. The result will be a flat cone or pyramid indistinguishable from the previous case.

In the case that there is a random distribution of dislocations a variety of phenomena occur, exhibited pictorially in Fig. 3 (1 to 12). In these pictures there is supposed to be positive supersaturation, so that each terrace makes a curve convex on its cliff side, running from one dislocation to another of opposite sense, or to the boundary of the crystal face. The supersaturation is supposed to be low, and fluctuating, so that in general



only one terrace-line—normally the longest—moves at a time. In each picture the terrace due to move next is specially marked. Shading indicates the area covered by fresh growth after picture 1. Among points to be specially noticed are :

(i) the holding up of one terrace-line behind another (pictures 2, 8, 9, 10) or at a dislocation connected to another terrace (9, 10, 11, 12) ;

(ii) the way in which a close pair of dislocations connecting a terrace facing the advancing terrace break it and so impede its passage (many examples, especially pictures 4, 5, 6). In these two ways, a dislocation pair holding a terrace facing *either* way is an obstruction to the passage of an advancing terrace ;

(iii) the way in which the obstructions of type (i) are broken down (10, 11, 4) ;

(iv) the relatively impregnable region in the bottom left-hand corner. In 6 growth into this region has ceased by obstruction of type (ii) : a new advancing terrace is held up again (at 9) by obstruction of type (i) : but this obstruction is more easily broken down, and by 12 all but a small portion of the crystal face has increased in thickness by at least one monolayer. A third advancing terrace, now commencing, will finally overrun this strong-point : however, a continuous line of closely spaced dislocations could be totally impenetrable below a critical supersaturation.

Obstruction of type (i) requires a little more consideration. One way in which type (i) obstruction can break down is the following. Each " pinned " terrace-line holds its equilibrium form by small statistical fluctuations back and forth. When two terraces lie together, the lower cannot fluctuate back under, nor the upper fluctuate forward over the other. Thus they exert a small effective repulsion on each other, and the lower one may be pushed beyond the critical curvature when the upper one arrives. Secondly, there is only a small region of seriously deformed crystal structure and hence of seriously reduced binding energy for new molecules, in the immediate neighbourhood of the dislocation. When the two terraces at such a point of obstruction face each other at an acute angle, as in pictures 9, 10, 11, only a small number of molecules need condense in these unfavourable positions to enable the terrace to link across and pass on. If the obstruction arises from a straight row of dislocations spaced  $l_1$  apart, this angle becomes smaller as  $l_0$  decreases, diminishing rapidly from  $120^\circ$  when  $l_0$  becomes less than  $2l_1$ . The obstruction will be ineffective when  $l_0$  is significantly smaller than this value. Even when it is greater, a comparatively small number of molecules (say, 6 to 10) in unfavourable positions suffice to overcome the obstruction, so that statistical fluctuations (negligible with regard to obstruction of type (ii)) can be effective. Thus obstruction of type (i) probably only imposes a delay rather than a total prohibition of growth.

In view of this, one might suppose it a particularly important question whether the numbers of right-handed and left-handed dislocation-ends in a face are equal or not : the surplus of one kind might have very long terrace-lines linking them to the boundary. This is not the case for reasonably uniformly or randomly distributed dislocations, unless the relative numbers are very different. If, in an area  $A$ ,  $n$  dislocations of one kind and  $m$  of another are formed randomly, like head and tail throws of a coin, the probable excess of one kind,  $n - m$ , is  $1.35 (n + m)^{1/2} \approx 1.9 n^{1/2}$  : but if it does not exceed  $\pi n^{1/2}$  (where  $n > m$ ) the dislocations linked to the boundary can all be close to it, and the longest necessary terrace inappreciably longer than in the case  $n = m$ , i.e., still of the order of magnitude  $(A/n)^{1/2}$ . In a systematically deformed crystal it is possible to have  $n \gg m$ , and then there is always a terrace-line at least as long as the  $(m + 1)$ th furthest dislocation of the  $n$  from the boundary.

The most important aspect of the type (i) obstruction by a fence of dislocations all of the same kind is that every boundary between two crystal blocks inclined at a small angle to each other constitutes such a fence (see Fig. 4, and Fig. 12 in ref. 9). The distance between the dislocations in the fence, measured in molecular spacings, is equal to the reciprocal angle of rotation between the two blocks, whether for "tilt," "twist" or more general boundaries, so long as the angle is small. A possible cause of the *visible* growth terraces sometimes seen advancing over a crystal face is that a number of molecular terraces have accumulated behind such a fence, and then been set free by a rise in supersaturation: but it is possible to think of alternative causes which can bring about the same "bunching" of molecular growth terraces into visible ones.

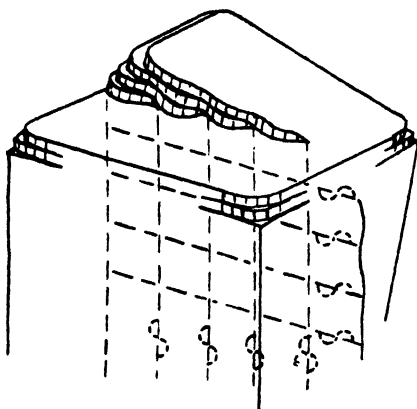


FIG. 4.—"Twist" boundary—a crossed grid of screw dislocations.

It remains to elaborate the concept of dislocations somewhat, to free it from the simplifications introduced by considering only simple cubic crystals, with cube faces. In the general case the important property of a dislocation is its displacement vector (or Burgers vector). If this vector has a component normal to the crystal face on which the dislocation line ends, there will be an associated molecular terrace in the face, promoting crystal growth. But there is also an important distinction between perfect dislocations, whose displacement vectors are lattice vectors, and imperfect dislocations whose displacement vectors lead in general from a lattice position to a twin-lattice position. Such is the dislocation with displacement vector  $\left(-\frac{1}{3}, -\frac{1}{3}, -\frac{1}{3}\right)$ , produced in the close-packed cubic crystal by omission of part of a (111) close-packed plane of atoms. This dislocation cannot glide,<sup>12</sup> but must lie in its (111) plane: ending in a (111) or similar surface, it attaches a molecular growth terrace in the usual way—but every time this growth terrace reaches the trace in the surface of the missing plane of atoms, the terrace must pause unless there is a definite supersaturation. At this boundary the lattice is not continuous but has a translation-twin relationship. Instead of the usual 6 contacts per added atom, characteristic of cubic close-packing, one row of atoms added at this boundary make 7 contacts each, and the next row only 5. The latter loosely bound row will only be formed at a definite supersaturation, or by statistical fluctuation after delay.

<sup>12</sup> Frank, *Proc. Physic. Soc. A*, 1949, **62**, 202.

## 54 INFLUENCE OF DISLOCATIONS ON CRYSTAL GROWTH

We must give a brief account of the origin of the dislocations which, it is suggested, dominate crystal growth. The chief origins which have been thought of so far are :

(i) Surface nucleation of layers in improper (e.g., twin) positions and proper positions simultaneously on the same face. Where these meet there is a dislocation. It must be remembered that the initial nucleation of the crystal always takes place at high supersaturation, much more than adequate for the Becker-Döring condition for surface nucleation on a perfect face.

(ii) Formation of one-dimensional dislocations in the edge row of the growing terrace (cf., van der Merwe's contribution to this Discussion). Such one-dimensional dislocations have an energy similar to the latent heat of evaporation of a molecule, and consequently exist in thermal equilibrium. During rapid growth at high supersaturation they can be trapped in an edge row, developing into two-dimensional and thence into three-dimensional dislocations.

(iii) The development of curvature in the growing crystal owing to the presence of impurities (a subject to be treated at length elsewhere). This ultimately leads to stress in the surface which demands a certain supersaturation for further growth, which can then continue if, and only if, dislocations are formed.

(iv) When *systems* of dislocations are present (particularly sub-grain boundaries) it is probable that the stress they would cause in perfect crystal compels the formation of further members of the system in growth : i.e., sub-grain *boundaries* are propagated in lineage structure.

(v) Aggregation of molecular vacancies into flat collapsed cavities (the edges of which are dislocation loops) whenever the temperature of a crystal is lowered. This is very likely the process responsible for the intensification of X-ray reflection (the so-called establishment of mosaic structure) when an organic crystal is plunged in liquid air.

(vi) Plastic yield under mechanical stress : this is believed not to create dislocations *ab initio* but to multiply those already present.<sup>11</sup>

These various considerations indicate that the initial dislocations necessary for growth are formed inevitably in the conditions needed for nucleation : and that to secure the best attainable perfection thereafter we must ensure small supersaturation (this involves good stirring, or there will be large supersaturation at the corners when it is small at the centre of a face), high purity of materials, steady temperature and absence of mechanical stresses.

The effect which dislocations have upon crystal growth produces a rather odd natural selection both of imperfection and perfection in crystals. The nucleation stage with high supersaturation makes a population of initial nuclei of varied, mostly rather great, imperfection. If growth is now carried out at low supersaturation, only a few of these seeds, in which dislocations are relatively far apart, will grow. The lower the supersaturation at this stage, the fewer and the more perfect the seeds which will actually grow. But the completely perfect crystal will not grow in any circumstances : the conditions which could cause it to grow would also soon make it imperfect.

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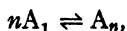
# KINETICS OF THE FORMATION OF NUCLEI AND STATISTICAL THEORY OF CONDENSATION

BY R. BECKER

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In the theory of the formation of nuclei<sup>1,2</sup> it has generally been assumed that in every gas there exist, in addition to single molecules ( $Z_1$  per unit volume), aggregates containing 2, 3, . . . .  $n$  molecules, and if  $n$  is not too small these aggregates can be regarded as spherical drops containing  $n$  molecules each. It may now be asked, what is the equilibrium number  $n$  of drops containing  $n$  molecules? Provided that the vapour is not supersaturated there is only one solution to this problem.

It is possible to treat the problem from a thermodynamic standpoint by considering the equilibrium,



or alternatively, kinetically by an examination of the rate of formation and disappearance of the number of drops containing  $n$  molecules. This may occur by means of an aggregate  $A_{n-1}$  taking up a molecule, or by an aggregate  $A_{n+1}$  losing a molecule. At equilibrium  $Z_n$  must be a constant. This kinetic method of approach is less exact than the thermodynamic method as it is necessary to make certain definite assumptions concerning the rates of evaporation and condensation. However, it is superior to the thermodynamic approach in that it is applicable to systems which are not in equilibrium. For example, it is possible to determine kinetically the rate of change of  $Z_n$  with time, and furthermore, the method leads to the solution of the problem of the frequency of formation of nuclei as a function of the degree of supersaturation.

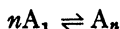
About twelve years ago Mayer<sup>3,4</sup> independently developed a statistical theory of condensation which must be regarded as one of the most important advances in statistical mechanics made in recent times. Mayer attacked the problem in a more general way by assuming that  $N$  molecules occupy a volume  $V$  and that between any pair of molecules there is a potential energy of interaction  $v_r$  which depends only upon the distance apart  $r$  and which rapidly tends to zero as  $r$  increases.

In an original discussion of the general integral of state Mayer shows that this may be expressed as a sum of terms represented by the symbols  $m_1, m_2, \dots, m_l, \dots$ ; this series indicates that  $m_l$  clusters containing  $l$  molecules are present. Even if the physical meaning of the clusters, which arises in Mayer's theory from purely mathematical considerations, is not altogether clear, it is important to note that at temperatures which are not too high and for values of  $l$  which are not too low, the  $m_l$  terms of Mayer are the same as the drop number  $Z_n$  of the earlier theory. Thus, fundamentally Mayer's theory indicates a method of calculation of  $Z_n$  and in particular for the derivation of a numerical factor common to all values of  $Z_n$ , hitherto uncalculated on previous theories. An important confirmation of this concept in Mayer's theory is obtained if the potential energy associated with the earth's gravitational field is introduced into the general partition function. It appears from this that individual clusters behave as particles of mass  $l$  times the molecular weight in relation to their distribution at various heights; i.e., the large clusters tend to sink to ground level.

A particularly brilliant aspect of Mayer's method is in its application to the Einstein treatment of the condensation of helium based on Bose statistics. The condensation is generally described as a difficultly conceivable process in the moment space but Uhlenbeck showed that it could be regarded as a true formation of clusters, analogous to that occurring for ordinary vapours in Mayer's theory.

Even if no energy other than kinetic energy is introduced into the partition function the application of the Bose statistics to helium gas shows that an effect exists which amounts to a tendency for mutual attraction between the atoms, comparable to a potential energy of interaction, and which leads ultimately to the formation of clusters distributed in a gravitational field in the normal way. Thus the Einstein condensation may be regarded as a particularly simple case of Mayer's theory which has the special advantage that all the integrals concerned may be completely evaluated. Unfortunately its significance is lessened by the fact that it cannot take into account the van der Waals' attraction between the atoms which is decisive for the observed condensation of helium.<sup>5 6</sup> The contribution of the theory to this particularly important phenomenon, which is so clearly related to the existence of helium II, is not so great therefore as had been hoped.

**The Chemical Equilibrium.** The methods outlined above may now be considered in somewhat greater detail. We shall consider first the reaction



as a chemical equilibrium according to van't Hoff's method.

Consider an equilibrium box in which there are  $Z_1$  single molecules per cm.<sup>3</sup> and  $Z_n$  drops each containing  $n$  molecules; let this box be connected to a vessel containing  $n$  mols of  $A_1$  at a concentration  $Z_1^\circ$ . We shall consider the work  $W$ , gained by a single molecule, when  $n$  mols of  $A_1$  at concentration  $Z_1^\circ$  are reversibly and isothermally mixed with one mol of the drops  $A_n$  at a concentration  $Z_n^\circ$ . If this process is carried out in the usual manner using semipermeable membranes, so that the reaction takes place within the equilibrium box, then

$$W = kT \left\{ n \log \frac{Z_1^\circ}{Z_1} - \log \frac{Z_n^\circ}{Z_n} \right\}.$$

This reaction may also be carried out by first compressing the  $n$  mols from the initial concentration  $Z_1^\circ$  to a concentration  $Z_{\text{sat}}$ . (that of the saturated vapour over a flat liquid surface), then condensing the vapour to a liquid, and finally by producing one mol of drops from the liquid, each drop having a surface area  $F_n$ . If in this last operation the drops are regarded as macroscopic entities, the work done is  $\sigma F_n$ , where  $\sigma$  = surface tension, and the total work gained at this stage in the process is therefore

$$kTn \log \frac{Z_1^\circ}{Z_{\text{sat}}} - \sigma F_n.$$

There now arises a difficulty which is characteristic of the whole problem. In the above considerations we have arrived at a stage where the drops, regarded as macroscopic entities, are situated adjacent to each other, whereas in the statement of the problem they were described as gas molecules existing at a concentration  $Z_n^\circ$ . Actually we have departed from a strict thermodynamic cycle by considering the individual drops as being formed from the liquid one by one. The following solution of the above difficulty may be suggested. If  $V_n$  is the volume of a drop, it is possible to consider the drops situated adjacent to each other as a gas of concentration  $1/V_n$ . If

this is permissible then the work gained in reaching the concentration  $Z_n^\circ$  is  $-kT \log Z_n^\circ V_n$ , hence

$$W' = kT \left\{ n \log \frac{Z_1^\circ}{Z_{\text{sat}}} - \log Z_n^\circ V_n \right\} - \sigma F_n.$$

As  $W = W'$  then

$$Z_n = Z_1^n V_n^{-1} \cdot Z_{\text{sat}}^{-n} e^{-\frac{\sigma F_n}{kT}}.$$

If  $p$ , the pressure exerted by the molecules, is  $Z_1 kT$  and  $p_\infty$ , the saturation vapour pressure, is  $Z_{\text{sat}} kT$ , then

$$Z_n = V_n^{-1} \cdot \left( \frac{p}{p_\infty} \right)^n e^{-\frac{\sigma F_n}{kT}}.$$

If we substitute  $K_n/n$  for the somewhat uncertain quantity  $V_n^{-1}$  then we have

$$Z_n = \frac{K_n}{n} \left( \frac{p}{p_\infty} \right)^n \cdot e^{-\frac{\sigma F_n}{kT}}, \quad (1)$$

in which the factor  $K_n$  depends on  $n$  in a manner which is not accurately known. The total number of molecules is

$$N = \sum_1^N n Z_n. \quad (2)$$

This series converges only when  $p < p_\infty$ . For  $p > p_\infty$ , however, it diverges.  $Z_n$  considered as a function of  $n$  has a minimum value and from this value the terms increase indefinitely. The minimum may be calculated in the following way. Let  $r_n$  = radius of a drop containing  $n$  molecules; then

$$V_n = \frac{4\pi}{3} r_n^3 = n \cdot v_{\text{liq}}.$$

( $v_{\text{liq}}$  = the specific volume of the liquid); hence  $F_n$  is proportional to  $n^{2/3}$  and therefore,

$$\frac{dF}{dn} = \frac{2}{3} \frac{F}{n} = \frac{2}{3} \frac{4\pi r_n^2 v_{\text{liq}}}{4/3 \cdot \pi r_n^3} = \frac{2v_{\text{liq}}}{r_n}.$$

From this we have

$$\frac{d \log Z}{dn} = \log \frac{p}{p_\infty} - \log \frac{p_n}{p_\infty} = \log \frac{p}{p_n}. \quad (3)$$

Here  $p_n$  is the equilibrium vapour pressure of a drop of radius  $r_n$ , i.e.,

$$\log \frac{p_n}{p_\infty} = \frac{2\sigma v_{\text{liq}}}{kT r_n}.$$

Therefore the series of numbers  $Z_n$  in (1) approaches so closely to zero for  $p < p_\infty$  that  $\sum n Z_n$  converges; for  $p > p_\infty$ , however, it may be seen from (3) that the series has a minimum value for that value of the drop size for which the vapour pressure  $p_n$  is just equal to the given vapour pressure  $p$ . In the latter case therefore equilibrium is impossible and the kinetic theory must be used.

**The Kinetic Treatment.** In the *kinetic* treatment we consider the growth and disappearance of the drop separately. Let  $a_0$  = the number of single molecules arriving per sec. per  $\text{cm}^2$  at the surface of the drop  $A_n$ , and  $q_n$  = the number of molecules which evaporate per sec. per  $\text{cm}^2$  from the surface of a drop  $A_n$ . The ratio  $a_0/q_n$  is equal to  $p/p_n$ , i.e.,

$$\beta = \frac{a_0}{q_n} = \frac{p}{p_n} e^{-\frac{2\sigma v_{\text{liq}}}{kT r_n}}.$$

The number of processes  $A_n \rightarrow A_{n+1}$  occurring per second is given on these assumptions as  $Z_n \cdot F_{n+1} a_0$ , and the number of processes  $A_{n+1} \rightarrow A_n$  as  $Z_{n+1} \cdot F_{n+1} \cdot q_{n+1}$ . The excess of the latter number over the former is designated by  $J$ , where

$$J = a_0 F_{n+1} \left( Z_n - Z_{n+1} \cdot \frac{1}{\beta^{n+1}} \right).$$

For the *equilibrium condition*,  $J = 0$ , and an expression essentially the same as (1) is obtained again for  $Z_n$ . If, however,  $p > p_\infty$  then all  $Z_n$  values for  $n > n_k$  may be placed arbitrarily equal to zero (i.e., any nuclei formed are removed). The quasi-stationary state where  $J$  is independent of  $n$  may then be considered. Thus it follows from eqn. (8) that by eliminating all the terms  $Z_2, Z_3, \dots, Z_{n_k}$  a value for  $J$  is obtained, and this may be called the frequency of formation of nuclei. Thus for example one obtains

$$J = \frac{a_0 K}{n_k} \sqrt{\frac{A}{3\pi}} e^{-\frac{A}{kT}},$$

where  $A = \frac{1}{2} \sigma F_k$ , the work which must be done isothermally and reversibly to produce one critical drop.

**Mayer's Theory of Condensation.** In this theory  $N$  atoms are considered with a potential energy  $v(r)$  dependent only on distance and the corresponding partition function  $\zeta$  is

$$h^{-3N} \int \exp \left\{ \frac{-1}{2mkT} (\xi_1^2 + \dots + \xi_N^2) - \frac{1}{kT} (r_{12} + r_{23} + \dots) \right\} d\xi_1 \dots d\xi_N dr_1 \dots dr_N.$$

If the integration is carried out with respect to the momentum then  $\lambda$ , the de Broglie wavelength at a temperature  $T$ , may be written as  $\lambda = h(2\pi mkT)^{-1/2}$ , and the term  $e^{-\frac{v_r}{kT}}$  as  $1 + f(r)$ . Then

$$\zeta = \lambda^{-3N} \int (1 + f_{12})(1 + f_{13}) \dots (1 + f_{jk}) \dots dr_1 \dots dr_N. \quad (4)$$

The  $f(r)$  values only differ from zero for small values of  $r$ . If therefore an integral such as

$$\int f_{12} f_{23} dr_1 dr_2 dr_3$$

has to be evaluated, then the integration may be performed firstly for a given  $r_1$  from 0 to  $\infty$  over  $r_1$  and  $r_2$ . Only the last integration over  $r_1$  contains the volume factor  $V$ . In separating the terms in (4), all those may be taken out in which, for example, the first three particles form a cluster at  $l = 3$ . These terms will be the ones containing the factors  $f_{12} f_{23}$  or  $f_{12} f_{13}$  or  $f_{12} f_{13} f_{23}$  and in which the indices 1, 2, 3 do not otherwise occur. From the sum of all these terms a factor

$$V 3! b_3 = \int (f_{12} f_{23} + f_{12} f_{13} + f_{13} f_{23} + f_{12} f_{23} f_{31}) dr_1 dr_2 dr_3 \quad (4a)$$

may be split out where the term  $b_3$  is defined so that it no longer contains the volume.

From the sum remaining after the elimination of this factor the terms containing  $f_{4,5}$  and in which the indices 4, 5 do not otherwise occur may be extracted. Proceeding in this manner  $\zeta$  may finally be split up into terms  $\zeta_{m_1, m_2, \dots, m_l, \dots}$ , where the individual sums are represented by:

$$\left. \begin{array}{lll} m_1 & \text{clusters containing 1 atom,} \\ m_2 & \text{,, ,, 2 atoms,} \\ m_l & \text{,, ,, l ,,} \end{array} \right\} \quad (5)$$

With  $b$  defined by

$$Vl!b_l = \int \{f_{12}f_{23} \dots f_{l-1,l} + \dots\} d\mathbf{r}_1 \dots d\mathbf{r}_l \quad (6)$$

for the contribution of such a series of terms,

$$\prod_l (Vl!b_l)^{m_l}$$

is obtained.

Now in general there are  $N! \prod_l \frac{1}{l!^{m_l} m_l!}$  possibilities of distributing the  $N$  particles in the clusters given by (5). The final value obtained for  $\zeta$  is therefore

$$\zeta = \frac{N!}{\lambda^{3N}} \sum_m \prod_l \frac{(Vb_l)^{m_l}}{m_l!} \quad (7)$$

The  $\Sigma$  is taken over all values of the series  $m_1, m_2, \dots$  which satisfy the condition  $\Sigma_l m_l = N$ . If one of the terms in the sum is relatively so great that for thermodynamic purposes it may replace  $\zeta$ , then the indices  $m_l$  of this term give the most probable values  $\bar{m}_l$  for the numbers  $m_l$  of the clusters containing  $l$  molecules.

The appropriate calculation gives

$$\bar{m}_l = Vb_l A^l, \quad (8)$$

where the value of the parameter  $A$  is given by the condition  $N = \Sigma_l m_l$ , i.e., by

$$N = V \sum_{l=1}^{\infty} l b_l A^l \quad (9)$$

Using this approximation

$$\log \bar{\zeta} = -3N \log \lambda + \log N! - N \log A + V \Sigma_l b_l A^l$$

is obtained. From this it follows that the pressure  $p = -kT \frac{\partial}{\partial V} \log \bar{\zeta}$ ,

$$\text{or} \quad p = kT \frac{\Sigma_l m_l}{V} \quad (10)$$

Hence the clusters introduced by (5) affect the pressure as independent particles of an ideal gas. Expression (9) is of particular interest to us because it gives the value of  $A$  as a function of the given density  $N/V$  of the substance. If  $A_0$  is the convergence limit of the power series  $\Sigma_l b_l A^l$ , then the finite sum of (14) assumes enormous values as soon as  $A > A_0$ . For increasing values of  $N/V$ , therefore,  $A$  can only increase up to this limiting value. For further increases of  $N/V$ ,  $A$  retains the constant value  $A_0$  and hence, from (8), the term  $\bar{m}_l/V$  has also a definite value, i.e.,  $b_l A_0^l$ . We are then in the region of condensation, where an isothermal diminution of volume only causes an increase in the amount of condensate but does not give rise to any change in the vapour phase. Also, as may be seen from (10), the pressure remains independent of the volume in this region. If the convergence limit  $A_0$  is introduced for the series (9) and if

$$Z_l = \frac{\bar{m}_l}{V}$$

where  $Z_l$  is the concentration of the clusters containing  $l$  atoms, then

$$Z_l = b_l A_0^l \left( \frac{A}{A_0} \right)^l.$$



From this equation a comparison may be made with the previous equilibrium eqn. (6) for spherical drops,

$$Z_n = \frac{K_n}{n} \left( \frac{p}{p_\infty} \right)^n e^{-\frac{\sigma F_n}{kT}},$$

by taking

$$\left. \begin{aligned} \frac{p}{p_\infty} &= \frac{A}{A_0} \\ b_n &= A_0^n \frac{K_n}{n} e^{-\frac{\sigma F_n}{kT}} \end{aligned} \right\} \quad \text{. . . . . (11)}$$

and

From (8) it is immediately seen that the parameter  $A$  signifies the concentration of the single molecules.

**The Bose-Einstein Condensation.** In this the potential energy is not at first considered. In the calculation of the partition function

$$\zeta = \sum_r e^{-\frac{E_r}{kT}}$$

for  $N$  helium atoms in a volume  $V$ ,  $E_r$  has the form

$$\frac{1}{2m} [\xi_1^2 + \dots + \xi_N^2].$$

If  $\varphi_r(r_1, \dots, r_N)$  is the symmetrical eigenfunction belonging to the energy  $E_r$  of the total system

$$\text{with} \quad \int |\varphi|^2 dr_1 \dots dr_N = 1,$$

then  $\zeta$  may be written in the form

$$\zeta = \int \sum_r e^{-\frac{E_r}{kT}} \varphi_r \varphi_r^* dr_1 \dots dr_N \quad \text{. . . . . (12)}$$

This sum, taken over all eigenvalues, may be accurately evaluated and gives the result

$$\sum_r e^{-\frac{E_r}{kT}} \varphi_r \varphi_r^* = \lambda^{-3N} \cdot \sum_P e^{-\frac{\pi}{\lambda^2} (|r_1 - r_{P_1}|^2 + |r_1 - r_{P_2}|^2 + \dots)}.$$

The summation must be carried out over all the  $N!$  permutations  $P$  of the spaces  $r_j$ . In a particular permutation, for example,  $r_1$  should be taken as  $r_{P_1}$ ,  $r_2$  as  $r_{P_2}$ , etc.

Thus, a single partial integral arising in this manner from (12) is

$$\int e^{-\frac{\pi}{\lambda^2} (r_1^2 + r_2^2 + r_3^2)} dr_1 dr_2 dr_3,$$

similarly to eqn. (4a) of Mayer's theory discussed above.

Each single permutation may be denoted by the number  $m_l$  of the cycles of length  $l$  which occur within it. In this case the appropriate integrals may be easily evaluated. Defining the value of  $b$  again, this time by

$$Vlb_l = \int e^{-\frac{\pi}{\lambda^2} (r_1^2 + r_2^2 + \dots + r_n^2)} dr_1 \dots dr_l,$$

we obtain

$$b_l = \frac{\lambda^{3(l-1)}}{l^{3/2}} \quad \text{. . . . . (13)}$$

With this value of  $b_l$ , the partition function given by (7) may be formulated, and hence the numbers of the corresponding clusters given by (8) can be

obtained. The saturation density may also be accurately calculated. For the series resulting from the combination of (9) with (13), i.e.,

$$\frac{N}{V} = \sum l b_l A^l = \frac{1}{\lambda^3} \sum \frac{1}{l^{3/2}} (A \lambda^3)^l,$$

there is a convergence limit at  $A \lambda^3 = 1$ , and in this case it yields the limiting value

$$\left(\frac{N}{V}\right)_{\text{lim.}} = \frac{1}{\lambda^3} \left(1 + \frac{1}{2^{3/2}} + \frac{1}{3^{3/2}} + \dots\right) = \frac{2.61}{\lambda^3}.$$

This corresponds to the well-known fact that the Einstein condensation begins when the single atoms have only the volume  $\frac{\lambda^3}{2.61}$  available to each of them. In this equation  $\lambda = h(2\pi m k T)^{-1/2}$ , i.e., the de Broglie wavelength corresponding to the temperature  $T$ .

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<sup>1</sup> Volmer, *Kinetik der Phasenbildung* (Dresden, 1939).

<sup>2</sup> Becker and Döring, *Ann. Physik*, 1935, **24**, 719.

<sup>3</sup> Mayer, *J. Chem. Physics*, 1937, **5**, 67; and subsequent volumes.

<sup>4</sup> Mayer and Goeppert Mayer, *Statistical Mechanics* (New York, 1946). In particular Chap. 13, 14.

<sup>5</sup> Born and Fuchs, *Proc. Roy. Soc. A*, 1938, **166**, 391.

<sup>6</sup> Kahn, *Dissertation* (Utrecht, 1938).

## GENERAL PRINCIPLES OF CRYSTAL GROWTH

BY PAUL H. EGLI AND S. ZERFOSS

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Certain facts of crystal growth have been observed with sufficient regularity to justify their being regarded as general principles. Some are familiar and will be mentioned only briefly for the sake of coherence. Others, equally significant, have received scant attention. A systematic consideration of this total body of facts should provide the background for extending the theories of crystallization.

Classifying the principles of crystal growth in a completely logical fashion is impossible because of the manner in which they are interrelated. Accordingly, some repetition will be necessary and the significance of certain experiments will require discussion in connection with several phases of the general problem.

### Nucleation

Nucleation is discussed as the first phase of the problem because it is the initial step in the overall process of crystallization; but most of the factors that control nucleation apply in the same manner to growth, and can be discussed more conveniently in that connection. Moreover, the important facts of nucleation are well known and need only to be described briefly.

(1) The rate of nuclei formation increases with supercooling. Tammann and others<sup>1</sup> demonstrated that in melts the nucleation rate reaches a maximum and decreases with further supercooling as diffusion becomes the controlling factor. In solutions such a maximum is difficult to measure and probably does not usually occur.

(2) An incubation period is recognized in growth from melts during which nucleation cannot be measured. In solutions, even when seeded, a metastable region of supersaturation is also recognized within which nucleation cannot be measured. Numerous investigators have found, in the phase diagram, well-defined regions with sharp boundaries beyond which nucleation was observable, and it seems reasonable to conclude that under certain conditions the rate of nucleation in solution increases extremely rapidly with a small increase in supersaturation. The "metastable region" principle is widely employed in the control of industrial crystallization processes and is a useful concept which will be referred to without apology even though the behaviour is more properly described as a rate phenomenon.

(3) The extent of the incubation period (or metastable region of supersaturation) can be changed appreciably by slight changes in composition of the system. It is particularly important to note that the metastable region can be greatly increased beyond that of a pure solution by the addition of small amounts of certain additives. This little-explored phenomenon is well substantiated for numerous compounds and will be further described in subsequent sections dealing with growth factors.

(4) The incidence of nucleation depends on the previous history of the system. The evidence supporting the existence of superheatability of nuclei appears overwhelming. The work of Tammann and others<sup>1</sup> with organic melts and with metals would seem sufficiently convincing, but the matter is still disputed. The fact that increasing the amount and time of superheating a system reduces the incidence of nucleation during subsequent supercooling is apparently accepted, but the opponents of the concept of superheated nuclei offer an alternative explanation—that it is insoluble impurities and not nuclei of the principle solid phase which the superheat destroys. This is an important argument in view of the fact that superheat in solids is not predicted in the lattice dynamics of Born or by the theories of melting as recently discussed by Mayer. Accepting the experimental results as evidence of superheated nuclei would also appear to be somewhat out of harmony with Frenkel's<sup>2</sup> concept of nuclei formation from embryo.

This question is difficult to settle by investigation of solution systems because of the experimental difficulties of observing the early stages of nucleation. Efforts have been made at the Naval Research Laboratory to obtain reliable data by means of heat effects, Tyndall effects, small-angle scattering of X-rays, etc.—all with little success. Qualitatively, however, it was the general conclusion of the several chemists concerned with growth of numerous crystals from solution that the existence of superheated nuclei was verified by their experiments on the preparation of saturated solutions. This work is mentioned because in solution systems an explanation based on impurities is extremely unlikely. The reagents were prepared with great care, and in several instances any remaining impurities were in amounts less than those detectable by ordinary analytical techniques.

(5) Nucleation is induced by the presence of foreign bodies and by agitation of the system. These well-known facts deserve to be listed in

<sup>1</sup> Tammann, *Aggregatzustände* (Leipzig, 1922).

<sup>2</sup> Frenkel, *Kinetic Theory of Liquids* (Oxford Univ. Press, 1946).

view of the frequent statements that nucleation always occurs for these reasons, or even stronger, that nucleation can never occur without such assistance. Again, these are difficult statements to disprove completely by experiment.

### Crystal Growth

Almost all the phenomena of crystal growth—inclusion of impurities, habit modification, the genesis of twins and flaws—can be resolved into problems of growth rate under certain conditions. Accordingly the growth principles are presented, for the most part, on this basis. There are certain important phenomena, however, for which the rate aspect is not a convenient viewpoint, and which thus necessitate a more complete description.

(1) Growth rate increases with increasing supersaturation (or supercooling) and with agitation. The ramifications of these facts are too well known to require elaboration except perhaps noting that diffusion is remarkably constant in all water solutions, and also that normally a moderate amount of agitation is sufficient to eliminate diffusion as a controlling factor.

(2) Different faces of a single crystal (under the same degree of supersaturation and agitation) grow at different rates. The rules governing this significant feature of the growth process have been the subject of refinement throughout the history of crystal research.<sup>3 4</sup> In general, crystals possess faces of low indices because of the differential bonding along the few principal directions within the lattice. This idea can be restated in terms of growth toward a minimum free surface energy.

(3) The difference in the growth rate of various faces becomes smaller as the overall rate is increased. This long-recognized fact has been demonstrated in a sufficient number of systems and under a sufficient variety of conditions that it can be safely regarded as a general rule.

(4) Flawed surfaces grow more rapidly (under the same degree of supersaturation and agitation) than corresponding surfaces without detectable faults. This is meant to apply to twin boundaries, veils, lineage, mosaic structure and presumably any other type of large-order defect. Elaboration of this point is helpful in explaining why defects are propagated and frequently induce additional defects during subsequent growth. At growth rates appreciably below the maximum which can be supported for good growth certain types of flaws lose their rate advantage and may be healed over.

(5) The maximum rate at which good growth can be obtained on a particular surface (the "critical" rate) decreases as the size of that surface increases. This highly significant fact has apparently received little attention, but the supporting data appear convincing. Yamamoto<sup>5</sup> demonstrated the phenomenon very clearly with NaCl on a microscopic scale. Investigations at the Naval Research Laboratory, particularly by A. A. Kasper, showed similar results for  $\text{NH}_4\text{H}_2\text{PO}_4$  grown under a variety of conditions and the effect has been observed qualitatively in the growth of numerous other crystals.

The existence of a "critical" rate dependent on size would appear to lead to the conclusion that in practice there is a limit in size to which a good single crystal of each compound can be grown. Experience would appear to bear this out. The possibility is also predicted that there will be crystals in which zones developed by growth of certain faces will invariably be bad whereas adjoining zones may grow well, and that the volume of poor material could be reduced or eliminated by reducing on the seed the size

<sup>3</sup> Wells, *Phil. Mag.*, 1946, **37**, 184.

<sup>4</sup> Bueger, *Amer. Miner.*, 1947, **32**, 593.

<sup>5</sup> Yamamoto, *Sci. Papers. Inst. Physic. Chem. Res.*, 1939, **35**, 228.

of the face which grows poorly (in  $\text{NaBrO}_3$  use a 110-cut seed rather than 111-cut seed). This also has been demonstrated.

It is suggested that this principle must also be more clearly recognized in crystallization theory. Presumably in an ideal system in which growth could be maintained at an infinitely slow rate the limit on size would disappear, but it is also possible that the necessary rate would be below that induced by normal fluctuations at equilibrium. Further implications of this feature of the growth process will be discussed in connection with the general problem of growth from the viewpoint of supersaturation.

(6) The critical growth rate in solution systems increases with increasing temperature. This may also be true for any system with two or more components when temperature is a variable. Discussion of this point is necessary because of frequent statements that crystals contain more defects when grown at high temperatures. It is an accepted fact that as the temperature of a crystal increases, whether grown at a high temperature or heated after growth, the number and activity of atomic-scale defects increase. This applies, however, only to vacancies and dislocations of single ions or atoms and does not pertain to large-scale order. In fact, the increased activity with temperature, particularly at the surface, tends to improve the large-scale perfection of the structure during the growth process<sup>2</sup>; experimentally, it has been found that increasing temperature favours the formation of perfect textures over the formation of spontaneous nuclei or the various types of large-scale flaws.

In addition to the foregoing list, there is a rarely mentioned feature of the growth process which deserves considerable discussion. No one has yet offered a satisfactory answer to the basic question of why some compounds crystallize readily and others very poorly; and yet there are some striking facts on which to base such a discussion.

The first point to be noted is that in aqueous solution systems compounds which grow readily are all quite soluble. In general, slightly soluble compounds are grown with great difficulty, and highly soluble compounds are grown with great ease. This correlation is far from perfect, however, so that simple solubility is not a sufficient specification for good crystal growth, and it is necessary to consider the state of association. Some evidence indicates that the critical rate for a given surface increases with increasing association of solute. This is first a statement in harmony with the familiar expression of theory—that the growth rate depends on the difference in the chemical potentials of a particle on the crystal surface and of one in the fluid phase. But the statement also implies something more—namely, that increasing association increases the advantage to formation of a perfect structure relative to the formation of flaws or spontaneous nuclei.

The implications of these statements can be more readily discussed in terms of supersaturation than from a strict rate viewpoint. As previously discussed in connection with nucleation, this viewpoint is not a rigorous approach in terms of the kinetics of the rate process, but is a valuable concept for the sake of clarity.

The problem then becomes one of determining the range of supersaturation which will induce only perfect growth, the additional degrees of supersaturation which will induce flawed growth of various types and, finally, the degree of supersaturation which will induce spontaneous nuclei.

The amount of supersaturation which will induce perfect growth, lineage, etc., is of course dependent on the configuration of each crystal surface available for growth. For the purposes of this discussion, however, this factor can be neglected by assuming that each crystal has some face which is relatively favourable for growth so that the supersaturation required for that growth is small relative to that required for spontaneous nuclei and is somewhat

less than required to initiate a flaw. On this basis compounds which are difficult to crystallize are those which form spontaneous nuclei with very small degrees of supersaturation so that the range which will induce growth but not flaws is vanishingly small. Easily grown crystals are those for which nuclei form only with considerable supersaturation so that there is a large range in which only perfect growth is obtained.

Returning to the original assertion that ease of growth increases with increasing association of the solute, it is desirable to consider the supporting evidence from the supersaturation viewpoint. Quantitative data are difficult to obtain because of the difficulty of measuring and rigorously describing the quality of a crystal, but even more because of the lack of quantitative data regarding the amount of association of various salts in highly concentrated solutions. Acceptable evidence is thus by necessity limited to obvious gross effects, but several compounds can be discussed for which the growth behaviour clearly supports the viewpoint expressed.

NaCl is a typical example of a compound which is soluble but highly dissociated in solution, and experience indicates that NaCl is virtually impossible to grow into a perfect crystal at any reasonable rate from pure solution. It forms copious nuclei with very small degrees of supersaturation.  $\text{HIO}_3$  is typical of compounds known to be highly associated in solution. Nuclei form only with considerable supersaturation, and large, perfect crystals are easily grown. Also typical of the associated solutes which support supersaturation and form crystals readily are a large number of hydrated salts. Additional significant evidence was demonstrated by growing benzil from water solution and various organic solvents. Growth was difficult in every solvent except benzene; from this related environment nuclei were formed only with considerable supersaturation and growth was excellent. Thus all the available evidence appears to be in harmony, but additional data are desirable.

Significant information can be derived from the remarkable effect of small concentrations of foreign ions. The growth of several dozens of compounds has been markedly improved by the addition of "impurities" to the solution. A typical case is NaCl which grows with great difficulty from pure solution but which grows readily from a solution containing Pb. The obvious effect is to greatly increase the range of supersaturation within which spontaneous nuclei do not form. Yamamoto<sup>5</sup> obtained data for several compounds and this work has been extended at the Naval Research Laboratory to many solution systems. The same phenomenon has also been demonstrated there in the growth of alkali halides from a melt and has been reported by one competent investigator to apply in growth by flame fusion. The rules for selecting the most effective additive are not yet entirely obvious. Heavy metal, multivalent ions in concentrations of less than 0.01 mol-% are frequently the best choice, though small concentrations of anything that, if present in larger amounts, would modify the habit appear to be helpful. If concentrations beyond the optimum are used, the habit is modified, flaws are induced and spontaneous nuclei occur more readily than from pure solution. When properly used, however, this is a very powerful tool. In one case, for example, it made possible the formation of a compound which cannot be precipitated from pure solution;  $\text{K}_4\text{MnCl}_6$  can be formed only by the addition of  $\text{Pb}^{++}$  to the solution. In a practical sense, the phenomenon is extremely valuable for increasing the efficiency of growth processes for single crystals and has in addition promising applications in industrial crystallization of fine chemicals. From a scientific viewpoint it promises to contribute valuable clues to the overall problem of nucleation and growth.

Space does not permit thoroughly justifying the choice of the foregoing

factors as being the most significant to interpretation of the overall problem. Obviously many interesting facts have been completely neglected. Habit modification, oriented overgrowths, inclusion of impurities and similar fields of research contribute valuable information but for the most part appear to be less directly necessary for consideration as part of the primary process of crystallization. The points which are discussed can hardly be ignored in even a qualitative theory that is to be of any value. The concept of a critical growth rate dependent on the size of the surface and the extent of association in the fluid phase, for example, appears to be a basic factor in the process. It is hoped that discussion in this manner of a rather loose body of facts may point out more clearly than would a neat mathematical expression the status of our present knowledge and the direction most profitable for future research.

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### GENERAL DISCUSSION

**Dr. F. C. Frank** (*Bristol*) said: In putting forward a structure-sensitive theory of crystal growth I can draw an analogy with the theory of the strength of solids. This is in practice at least a hundred times less than their theoretical strength. So also crystals show a "growth resistance" which is a hundred times less than their theoretical growth resistance. We owe to Volmer, to Farkas and to Becker and Döring the recognition and rather difficult calculation of this theoretical growth resistance of a perfect crystal. It appears from their work that new deposition will not occur at an observable rate on a completed habit-face of a crystal unless the supersaturation exceeds a substantial critical amount. This is of the order 50 % for a typical crystal growing from the vapour—greatly in excess of any critical supersaturation for growth which has been observed. We lack data for quantitative calculation of crystals, particularly ionic crystals, growing from solution, but every small crystal which grows in polyhedral shape demonstrates the existence of a substantial "growth resistance." The transport of material to a sufficiently small crystal in solution is governed by the diffusion equation. The solution of this differential equation will be uniquely defined if *either* the concentration or the flux at the boundary is defined. There is no degree of freedom left to satisfy boundary conditions in terms of both. The growth of the crystal determines a boundary condition in the flux, and the concentration at the boundary is determined accordingly, and is necessarily non-uniform over the surface of a polyhedral crystal. The flux and concentration can only both be uniform at the surface of a sphere. The surface supersaturation being non-uniform and nowhere negative is at places substantial; namely, at the corners, where it reaches several per cent. in the experiments of Humphreys-Owen and Bunn. Thus the very fact that at least some crystals will grow polyhedrally without becoming dendritic shows that they have in places a substantial "growth resistance": but at the same time the maximum supersaturation such crystals will withstand all over, without growth occurring somewhere, appears experimentally, so far, to be less than 1 %.

**Dr. S. P. F. Humphreys-Owen** (*London*) said: Dr. Frank says that nucleation at a dislocation, or system of dislocations, is capable of undergoing discontinuous changes. But why should one system be in some way peculiar in that it produces the Nernst mode of growth, i.e., with equilibrium concentration at the face centre? Other systems appear to produce rates of growth which are less, and

which are associated with a concentration at the face centre which is above the equilibrium value. This indicates the onset of a resistance to growth. Again, how can a system of dislocations, which, according to Dr. Frank, has a considerable degree of persistence, permit the complete stoppage of growth which is sometimes observed in high supersaturation? Theories such as Dr. Frank's, which provide the crystal with a means of overcoming the difficulty of growth of a complete surface, appear to go too far in the other direction; they make growth too easy.

**Dr. F. C. Frank** (*Bristol*) said: The rate of growth, once in the range of conditions at which growth proceeds steadily, is very largely independent of the density of dislocations. To see why this is so, we must first consider the effect of a single dislocation. The attached growth front (terrace edge or step-line) winds itself up into a rotating spiral (Fig. 5). A pair of dislocations emit growth fronts in the form of closed rings with about the same spacing, provided the supersaturation is sufficient for them to be effective at all (Fig. 6). Now, growth

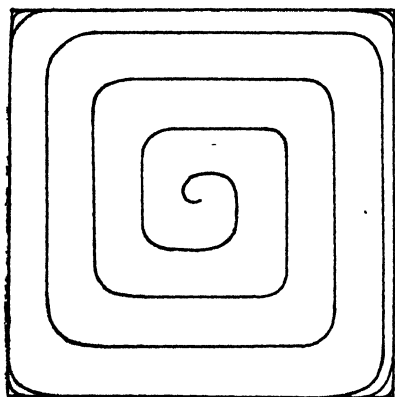


FIG. 5.—Spiral growth front attached to a single dislocation.

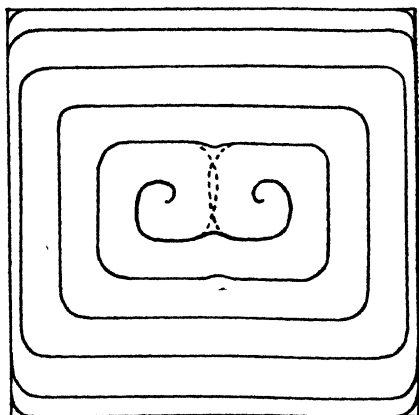


FIG. 6.—Development of growth fronts in closed loops from a pair of dislocations.

fronts differ from wave fronts in the fact that when they meet they annihilate. Hence if two different dislocations or groups of dislocations in the same crystal face are equally active in emitting growth fronts, the number of growth fronts passing any point in the face is the same as if either were active alone. A group of dislocations is as active as its most active member, and the growth rate of the whole face is determined by its most active dislocation group. All other groups yield within a short while to the domination of this most active group, and merely pass on the growth fronts received from it with a slight delay and in slightly modified form. The members of a group are nevertheless able to stimulate each other to somewhat enhanced activity. This is because a concave growth front, which is formed each time a pair of fronts meet, travels faster than normal, and so "helps the growth spiral round." This stimulation occurs at a supersaturation somewhat above the critical supersaturation for the group, below which they are inactive. If the supersaturation is increased considerably above this, the component dislocations then behave as though independent of each other; but this may be the right supersaturation for stimulation in some other group, which will then become dominant. An exact calculation is not easy, but it does not seem likely that this "stimulation" will make a much larger difference than a factor of 2.



Thus, at least for order of magnitude, the problem of calculating the growth rate of a face with any number of dislocations can be reduced to that of calculating the rate of growth based on a single dislocation: which is simply the rate of generation of fresh turns of the growth front spiral multiplied by the thickness of a molecular layer. As explained in my paper, this rate is proportional to  $v_\infty/l_0$ , where  $v_\infty$  is proportional to and  $l_0$  inversely proportional to  $\sigma_1$ , the supersaturation at the crystal surface in the neighbourhood of the dislocation or dominant dislocation group. Let us say the growth rate is  $w = A\sigma_1^2$ . But the controllable supersaturation  $\sigma_0$  is that at some point away from the crystal, separated from it by a diffusion barrier, as a result of which there is a drop in supersaturation  $(\sigma_0 - \sigma_1)$  proportional to the growth rate: say,  $(\sigma_0 - \sigma_1) = Bw$ . In consequence a growth rate establishes itself such that

$$AB^2w = AB\sigma_0 + \frac{1}{2}(1 - \sqrt{1 + 4AB\sigma_0}).$$

Briefly, the growth rate is proportional to the square of the supersaturation when this is small; but at high supersaturation the diffusion barrier takes

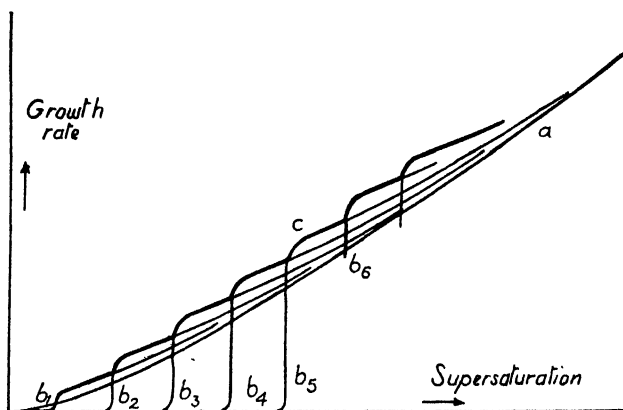


FIG. 7.—Crystal growth rate as a function of supersaturation (with simple diffusion barrier):—

- (a) for a single dislocation.
- ( $b_1$ ), ( $b_2$ ) . . . for variously spaced pairs or other groups of dislocations.
- (c) Resultant growth rate curve when all of the groups ( $b_1$ ), ( $b_2$ ) . . . are present.

control and "linearizes" the growth rate. This is represented by curve (a) in Fig. 7. Here we have assumed the simplest possible sort of diffusion barrier. In growth from a dilute vapour we actually have a more complex situation, with surface diffusion and molecular transport through the gas in "series-parallel" connection. This problem has been treated by Cabrera and leads to a curve in which the transition from the quadratic to the linear law of growth rates involves a region of reversed curvature.

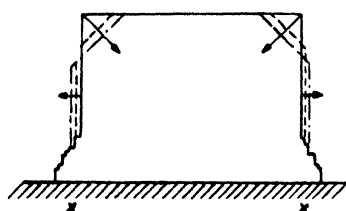
Now, if there are various dislocation groups in the crystal face, which would separately produce growth rate curves ( $b_1$ ), ( $b_2$ ) . . ., the resultant growth rate curve is  $c$  made up of the curves of the groups dominant at various supersaturations. Change of supersaturation can change the dominant group, thus changing the centre of the growth pyramid; but the growth rate is not greatly dependent on the dislocation structure once the supersaturation exceeds the critical supersaturation of the least growth-resistant dislocation group.

In further discussion with Dr. Cabrera and Mr. Burton, since the meeting, we have arrived at the conclusion that there is no existing experimental evidence of a critical supersaturation which necessarily indicates that dislocations are close together: the quadratic growth law which applies at small supersaturation when dislocations are far apart suffices to explain the existence of a supersaturation below which the growth rate is too small to be measured by the techniques which have been applied, as in the experiments of Volmer and Schultze, and of Nitschmann and Spangenberg.

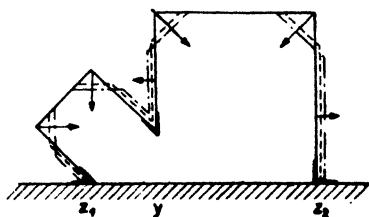
**Prof. I. N. Stranski (Berlin)** said: Hitherto it has been accepted that in (microscopically) visible crystals a spontaneous alteration of form is not possible in a state of thermal equilibrium. The reason for this has been recently seen to lie not primarily in the smallness of the relative vapour pressure differences between the various crystal faces, but rather in the fact that the building-up of new lattice planes is connected with the work of formation of two-dimensional nuclei, which according to Volmer tends to infinity with decrease of saturation.

In the experiments on urotropine the work of formation of two-dimensional nuclei is considerably decreased by the presence of re-entrant edges. In the accompanying Figure three types of re-entrant edges are seen to be active:

- (i) Mono-crystalline re-entrant edges formed by incompletely grown lattice planes,  $x$ .
- (ii) Poly-crystalline re-entrant edges formed by contact between two differently oriented individual crystals,  $y$ .
- (iii) Heterogeneous re-entrant edges formed by participation of the crystal substrate.



— Growth form



— Equilibrium form  
in vertical section

According to experiments by Honigmann, the mono-crystalline re-entrant edges  $x$  are particularly effective. The work of nucleus formation can only be purely one-dimensional at such defects, that is, even for very small supersaturations it remains negligible.<sup>1</sup> The relative vapour pressure difference  $\Delta P/P$  has been estimated as  $10^{-4}$  to  $10^{-3}$ , and for a fraction  $\theta$  of coverage by the adsorbed layer, for which the comparison is particularly valid, very reasonable values (very much less than 1) are obtained.

In all these experiments the crystals of urotropine are surrounded only by their own vapour. The admixture of foreign gases increases the times for the transfer of matter enormously.

**Dr. H. K. Hardy (Stoke Poges)** said: If, as Dr. Frank<sup>2</sup> has indicated, crystals grow by "winding themselves up" with screw dislocations, would it be correct to speak of their solution as an "unwinding" process? I am prompted to ask this because there was a paper by Bloch, Brings and Kuhn<sup>3</sup> in which the rate of solution of a crystal was taken as proportional to the edge length. On this hypothesis smaller crystals melted more slowly than large ones and this was taken as the source of undissolved crystal nuclei at temperatures not far from the melting point. If, instead of edge length, we substitute effective dislocation length as the criterion for the rate of solution, a stage will eventually be reached during solution (or melting) at which this is reduced to zero but at which a very small crystal fragment remains. This might be expected to have considerable stability and would form a source of crystal nuclei on subsequent cooling.

I would now like to mention some phenomena connected with precipitation in solid solutions since, by quenching from a high temperature, the effects of very high degrees of supersaturation can be studied when the alloy is allowed to

<sup>1</sup> See, for example, Stranski and Kaischew, *Ann. Physik*, 1935, **23**, 330.

<sup>2</sup> This Discussion.

<sup>3</sup> Bloch, Brings and Kuhn, *Z. physik. Chem. B*, 1931, **12**, 415.

decompose at a lower temperature. The most convenient starting point is the free energy-composition curve (Fig. 1). The equilibrium phases have the compositions given by the common tangent. Between the points Y-Y, the curve is concave to the composition axis and in this region  $\partial^2 F / \partial x^2$  has a negative value. In agreement with diffusion theory, fluctuation theory and the shape of the curve, we should expect that an alloy quenched to this region would show segregation of like atoms and possibly pre-precipitation effects associated with this. This is the case in the  $\text{Cu}_3\text{FeNi}_3$  alloy investigated by Daniels<sup>4</sup> in which variation of the ageing temperature changed first the degree of segregation and then the distance between segregates.

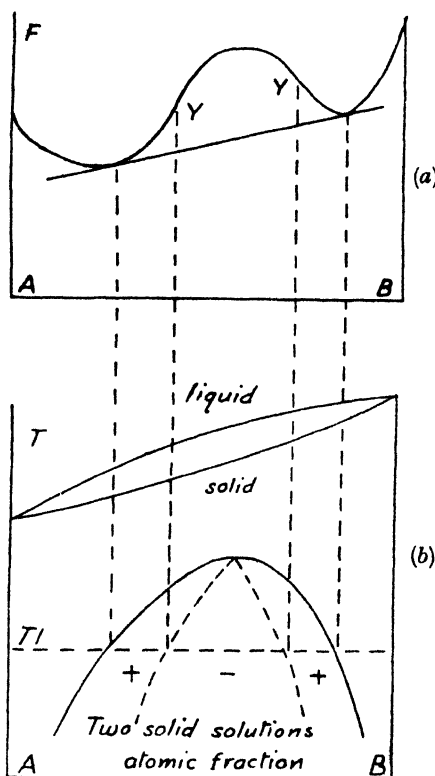


FIG. 1.

- (a) The free energy composition curve at  $T_1$  for the hypothetical equilibrium diagram in Fig. (b)  
 (b) The supersaturated solid solution has been divided into two regions corresponding to the inflections on the free energy composition curve and positive and negative values of  $\partial^2 F / \partial x^2$ .

In general, the solute atoms collect on preferred planes as "platelets" or even as "stringlets,"<sup>5</sup> but in some alloys the effects are more complicated than can be predicted by simple segregation. For example, in Al-Cu alloys this segregation requires nucleation, there being a critical nucleus size dependent on the ageing temperature. If we consider the Al-4 % Cu alloy, the segregation of solute atoms at room temperature after quenching from 530° C leads to an increase in hardness (Fig. 2). If the alloy is now raised to 200° C for a few minutes,

<sup>4</sup> Daniels, *Proc. Physic. Soc.*, 1948, **192**, 575.

<sup>5</sup> Geisler and Hill, *Acta Cryst.*, 1948, **1**, 238.

the segregates are dispersed and it reverts to its hardness after quenching. It will then re-age as before and the process can be repeated for at least 10 times and probably indefinitely (Fig. 2). The segregates (or Guinier-Preston zones as they are called) formed at room temperature are below the critical size for their growth at 200° C. This means that the free energy of such zones, even though not a separate phase, possesses a strain energy term proportional to their surface area, and hence their free energy will show a maximum when plotted against their radius (Fig. 3).

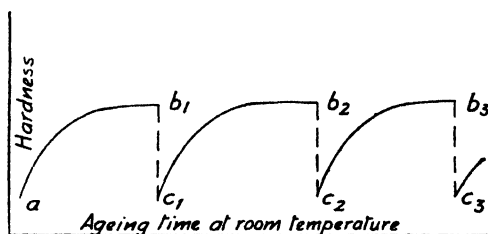


FIG. 2.—Behaviour of Al-4 % Cu alloy on natural ageing following quenching  $a-b_1$ , treated 5 min. 200° C to disperse the segregates of copper atoms  $b_1-c_1$ , etc., which re-establishes its ability to natural age  $c_1-b_2$ , etc.

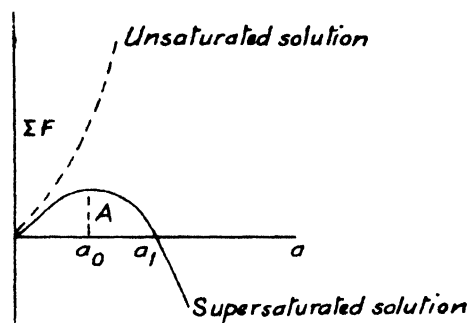


FIG. 3.—Variation of  $\Sigma F$  with size of nucleus.

Both Becker<sup>6,7</sup> and Borelius<sup>8,9</sup> have put forward theories to account for the rates of precipitation. Fig. 4 gives experimental curves on Pb-Sn alloys, where the logarithm of the time for half the resistance change associated with precipitation has been plotted against the reciprocal of the isothermal precipitating temperature in °K (the thick lines in Fig. 4). The arrows mark the temperature, calculated by Borelius<sup>9</sup> at which  $\partial^2 F / \partial x^2$  changes sign, at higher temperatures; where this is positive the rate of precipitation slows down. In Becker's theory<sup>7</sup> the rate of precipitation per unit volume of untransformed matrix is proportional to

$$e^{-(Q+A)/RT},$$

where  $Q$  is the activation energy for diffusion and  $A$  the activation energy for nucleation. I have applied this to the experimental results given in Fig. 4. The free energy terms needed were calculated using the methods of Borelius<sup>9,10</sup> and comparing them with Becker's equations.<sup>7</sup> By this means the activation energy was calculated for the initial precipitation. This leads to the dotted

<sup>6</sup> Becker, *Z. Metallkunde*, 1937, **29** (8), 245.

<sup>7</sup> Becker, *Ann. Physik.*, 1938, **32**, 128.

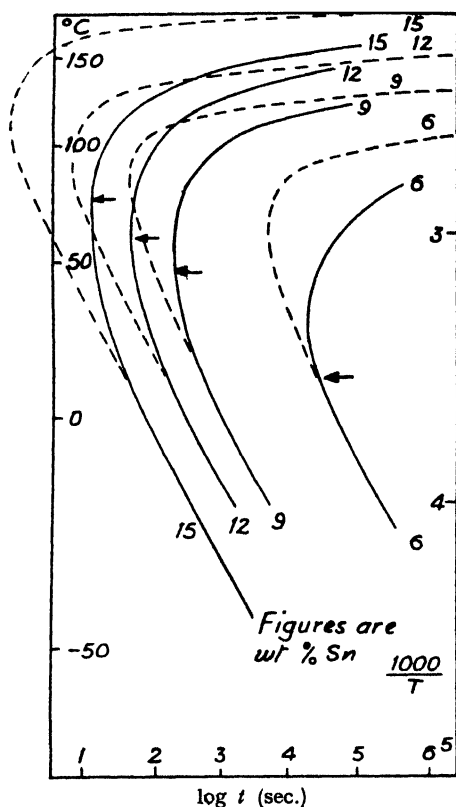
<sup>8</sup> Borelius, *Ann. Physik.*, 1938, **33**, 517.

<sup>9</sup> Borelius, *Arkiv. Mat. Fysik.*, 1945, **32** (1).

<sup>10</sup> Borelius, Larris and Ohlsson, *Arkiv Mat. Ast. Fysik. A*, 1944, **31** (10).

curves in Fig. 4. The agreement with experiment is not unreasonable in view of the simplifying assumptions which were made. If a correction were made for the decrease in rate of nucleation due to change in degree of supersaturation during precipitation, the theoretical curves would be moved to the right, i.e., closer to the experimental values.

I hope to publish a more detailed account of this work on precipitation in terms of the free energy composition curve at a later date.



$t$  = time to half resistance change.

← temperature for change from positive to negative value of  $\partial^2 F / \partial x^2$ .

Full lines experimental, dotted lines calculated.

FIG. 4.—Comparison of experimental curves for log time to half the resistance change of Pb-Sn alloys on ageing, plotted against  $\frac{1}{T}$ , and curves calculated from nucleation theory based on the original concentrations.

$$Q = 10,400 \text{ cal./g.-atom.}$$

**Dr. F. C. Frank** (*Bristol*) (*communicated*): Dissolution on a habit-face of a crystal with protected edges, at moderate subsaturation, should proceed in a manner closely equivalent to growth on fully developed habit-faces, i.e., by unbuilding at molecular terrace lines ending on screw dislocations, and there should be a critical subsaturation for dissolution, equal to the critical supersaturation for growth. But if the crystal edge is exposed to attack, it is a permanent source of terraces, and the dislocations then play no essential part in the steady-state dissolution process. Terraces attached to dislocations would

be involved in the initial attack, and would, I think, upset the proportionality between rate of attack and length of crystal edge; which could in any case only apply to initial conditions, since crystals in dissolution rapidly cease to be simple polyhedra and become bodies bounded by curved or rough surfaces, open to attack all over. The rate of steady-state dissolution is governed simply by transport of heat or matter.

It is theoretically possible to dissolve a crystal until the remaining fragment is undislocated, and, if now submitted to moderate supersaturation, can only grow out to the circumscribing polyhedron of habit-faces and then stop growing. I doubt whether this has practical importance, or will be easy to observe.

The paper of Bloch, Brings and Kuhn quoted is interesting but illogical. There is no reason why the edge-row ( $d$  in their Fig. 1) should be less easily attacked than the step-row ( $b$ ), or the corner atoms of the crystal less easily than those at "reproducible points" ( $c$ ). Actually, I know of no real evidence of any portion of a solid failing to melt above its melting point, and believe that "persistent nuclei" in metal melts are to be explained in a relatively trivial way, as foreign matter, e.g., oxide: the surmise of Horn and Masing<sup>11</sup> for antimony, that the impurity gradually dissolves in the melt, but is precipitated out on solidification, accounts for the details of behaviour, and may well apply in other cases.

**Mr. W. K. Burton and Dr. N. Cabrera** (*Bristol*) (*communicated*): With reference to the theory of crystal growth from the vapour, when there is an adsorbed layer of molecules of high mobility, the present situation can be summarized in the following way.

Let  $D$  be the diffusion coefficient of the adsorbed atoms and  $\tau$  their mean life on the surface (mean time between their condensation from the vapour and their evaporation again into the vapour). Then the mean displacement of adsorbed atoms is

$$\bar{x} = \sqrt{D\tau} = a/\beta,$$

where  $a$  is the interatomic distance and  $\beta$  is defined by formula (3) in Part II of our paper. For materials of low sublimation energy like iodine, we expect  $\bar{x}$  to be of the order of  $10^2$  interatomic distances, at room temperature. It will be much higher for metals.

If  $\bar{x}$  is bigger than the mean distance  $x_0$  between Kossel-Stranski-Frenkel kinks (places where condensation into the body of the crystal is easy), then it can be shown that the rate of growth of the crystal is proportional to the supersaturation  $\sigma = \alpha - 1$  (formula (6) in Part II of our paper). This formula is in good agreement with the linear law observed by Volmer and Schultze<sup>12</sup> on iodine, phosphorus and naphthalene at  $0^\circ\text{C}$ . These authors observed a linear law in all cases, but for iodine the actual rate of growth is smaller than the linear law, below supersaturations of the order  $10^{-2}$ .

The problem is to explain why there seems to be always such a high concentration of kinks. It is now quite clear that the concentration of kinks in a perfect habit face will be negligible, unless the supersaturation is very high (see our papers), and also that the only explanation for the observed growth at low supersaturations is the fact that the crystals are imperfect and the screw dislocations terminating in the surface (see Frank's paper) provide the required steps with a high concentration of kinks. The step between two dislocations of different sign will contribute to the growth if the distance  $d$  between them is bigger than the size  $l_0$  of the critical two-dimensional nucleus, which is of the order  $a/\sigma$ . If  $N$  is the number of dislocations per  $\text{cm}^2$ , then  $d \sim N^{-1/2}$ .

Now from the point of view of the rate of growth we can distinguish two cases: either the distances  $d$  between dislocations are bigger than the mean displacement  $\bar{x}$  defined above, or they are smaller. In the first case,  $d > \bar{x}$ , we expect two different critical supersaturations. Increasing the supersaturation  $\sigma$  from zero, there will be a first range where no observable growth will occur, up to  $\sigma = \sigma_1 \sim q/d$ . At this first critical supersaturation  $\sigma_1$  the growth will start more or less suddenly (see our paper, Part II).  $\sigma_1$  will be different from one crystal to another, and will

<sup>11</sup> Horn and Masing, *Z. Elektrochem.*, 1940, **46**, 109.

<sup>12</sup> Volmer and Schultze, *Z. physik. Chem. A*, 1931, **156**, 1.

be observable if it is of the order of, or bigger than,  $10^{-8}$ . Actually  $\sigma_1 \sim a/d \sim aN^{1/2}$ , therefore it will be observable if  $N$  is bigger than  $10^{10}$ . When  $\sigma > \sigma_1$ , Frank has shown that a dislocation or small group of dislocations will be the centre of a "growth pyramid," the distance between different step-lines of the pyramid being of the order of  $l_0$  or  $a/\sigma$ . When  $\sigma < \sigma_1 \sim a/\bar{x}$ , the rate of growth of the surface is more or less proportional to  $\sigma^2$  and is smaller than that given by the linear law. Finally, when  $\sigma > \sigma_2$ , the parabolic law goes over to the linear law, because the distance between step-lines of the pyramids is smaller than  $\bar{x}$  and we are under the conditions where the linear law holds. The second critical supersaturation  $\sigma_2$ , defined by  $\sigma_2 \sim a/\bar{x}$ , will be the same for all crystals,  $\bar{x}$  being independent of the imperfection of the crystal. This seems to be the case in the experiments on iodine by Volmer and Schultze.<sup>23</sup> Assuming, then, that the critical supersaturation observed in their experiments corresponds to  $\sigma_2$ , one can deduce the value  $\bar{x} \sim 10^3 a$ , which agrees with the expected value. The rate of growth for  $\sigma_1 < \sigma < \sigma_2$  will depend essentially on the distribution of dislocations.

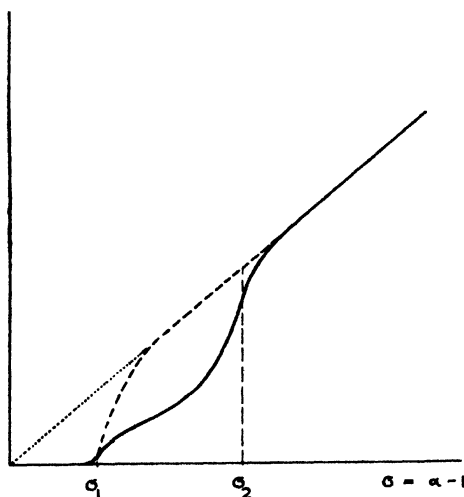


FIG. 1.

In the second case,  $\bar{x} > d$ , the first critical supersaturation only appears. We should expect the rate of growth to go over to the linear law rather suddenly (as represented by the dotted curve in Fig. 1) and the critical supersaturation to be different from one crystal to another. This situation would occur probably in metals, provided the crystal contained a number of dislocations high enough for the critical supersaturation to be observable.

**Prof. I. N. Stranski** (*Berlin*) said: Foreign (just as lattice) adsorbed molecules influence the growth and dissolution processes in two ways. Firstly, they favour (i.e., catalyze) the elementary processes by lowering the amount of energy required. In this connection we may consider the simple electrostatic model of the ionic crystal. To remove one adsorbed ion from a (001) face of a sodium chloride lattice an energy  $\phi_1 = 0.06601$  (in  $e^2/\gamma_0$  units) is required. To remove a single ion which is part of the same face an energy  $\phi_2 = 1.68155$  is necessary. To remove an adsorbed ion together with the ion of opposite charge (directly beneath it) an energy  $\phi_{1/2} = 0.74757$  will be needed. (This latter is the energy of separation of a molecule from the crystal boundary, involving only half-crystal forces.) The first process is naturally the most frequent. The third process can, however, occur much more frequently than the second, which is the relevant point here.

The adsorbed foreign molecules, however, retard the diffusion in the adsorption layer itself and in this way they may easily outweigh the first effect.

**Mr. R. S. Bradley** (*Leeds*) (*communicated*): A possible experimental test of Prof. Stranski's theory of surface polarization is the measurement of the heats of adsorption of the inert gases on ionic crystals, in comparison with the values calculated by the application of Prof. Stranski's theory.

**Prof. I. N. Stranski** (*Berlin*) said: Frank's idea is significant. It largely coincides with the views of Honigsmann and myself. In our preliminary communication<sup>13</sup> we also draw attention to the close connection between the processes occurring on the urotropine crystal with the growth-processes for twins, where likewise a considerable reduction in the work of formation of the two-dimensional nuclei on the boundary lines must be anticipated. We have also considered attributing the unsuccessful experiments of Volmer and Schultze (naphthalene, phosphorus) to the presence of re-entrant edges.

**Dr. J. L. Amorós** (*Barcelona*) (*communicated*): It is known from the so-called Donnay-Harker's law that the equilibrium form of crystals depends upon the real symmetry of the crystal, i.e., the group symmetry. This law has been criticized by me during the last few years. However, in my opinion the law remains valid in all respects because the crystal habit is directly related to the crystal structure.

The relation between structure and both growth and final form of the crystal appears to be clear when the stability of crystal body is realized. That stability is reached when the crystal faces are stable and the latter can only be stable when the co-ordination polyhedron or the close-packing of molecules is easily obtained.

Therefore the crystal growth must take place on those faces where either the completion of the co-ordination or the close-packing of molecules can be more easily obtained. As the crystal structure determines the symmetry of the cell, the crystal habit is likely to be a function of the space group elements of symmetry. Bearing in mind the above, it should be interesting to know the actual relationship between Stranski's theory and the Donnay-Harker's law.

**Dr. C. W. Bunn** (*I.C.I., Plastics*) said: The suggestion in Frank's paper that dislocations play a dominant part in crystal growth from vapour or solution arises from the conclusion that a perfect crystal bounded by low-index surfaces probably would not grow at all unless the supersaturation of the vapour or solution were very high. But the fact that crystals do grow at quite moderate supersaturations might be explained in two ways—either by assuming the presence of dislocations, or by assuming that the surfaces are not low-index surfaces. The observations described by myself and Emmett in this Discussion show that on many crystals growing rapidly from solution deposition takes place on the edges of spreading layers, and that these edges are, on the scale which can be observed in the optical microscope, high-index surfaces. If all the deposition surfaces are high-index surfaces on the molecular or ionic scale, there would appear to be no need to invoke dislocations to explain the continuance of growth, because even a crystal of perfect structure will grow readily if it has high-index surfaces. Our observations suggest that the central problem of crystal growth is the study of the factors which maintain high-index surfaces—the factors which determine that molecules are deposited in such a way that the new surface is again a high-index surface. There is a tendency for high-index surfaces to “heal”—i.e., for depositing molecules to form a low-index surface; what is it that prevents complete healing?

Our observations also show that layers usually spread outwards from the centres of crystal faces. Is there any reason to suppose that dislocations would occur preferentially at face centres? The electron microscope photograph of Wyckoff's<sup>14</sup> to which we refer in our paper (a protein crystal grown from aqueous solution) does not show a screw dislocation, or indeed any central structural imperfection in the top layer. It is true that this is not a photograph of an actually growing crystal, but of a crystal whose growth has been arrested, so it must be regarded as suggestive rather than conclusive, for we do not

<sup>13</sup> Stranski *et al.*, *Naturwiss.*, 1948, **35**, 156.

<sup>14</sup> Wyckoff, *Acta Cryst.*, 1948, **1**, 277.



know what might have happened after growth ceased. But it corresponds so exactly to what has been observed dynamically on a larger scale that it is difficult to resist the impression that it is a molecular growth-picture, and therefore deserves consideration.

The screw dislocation has been introduced because it is self-perpetuating; but it seems to me a rather special sort of imperfection, and I cannot visualize how it arises. I should feel happier about an imperfection theory based on simple strains, cracks and dislocations arising continuously and spontaneously perhaps as a result of thermal strains originating in the flow of heat of crystallization and depending on previous growth rates.

**Dr. F. C. Frank** (*Bristol*) (*partly communicated*): Prof. Stranski says, rightly, that growth at low supersaturation can occur on uncompleted molecular layers; but then, on a perfect crystal, uncompleted layers must finally become completed ones. From discussion with others, it appears necessary to say that also in other language: that high index surfaces grow readily, but thereby grow out, leaving low index surfaces. The essential importance of dislocations is that they prevent this happening, enabling the uncompleted layers or high index surfaces to persist.

If Wyckoff's Fig. 7, shown by Dr. Bunn, were a picture of a crystal growing from solution of low supersaturation, I should be rather worried. It is not: from Wyckoff's own description, it is a preparation made by smearing the crystalline pellet (formed by ultracentrifugation) on a glass surface.

I should like to reply to two more points raised by Dr. Bunn and one raised by Dr. Humphreys-Owen.

Firstly, is there any reason to suppose dislocations would occur preferentially at face centres? Yes, dislocation lines are effectively under tension. Given the chance they will usually make themselves as short as possible, and this will, as a rule, overcome any tendency of the dislocation line to adhere to a preferred direction in the crystal, when these requirements are in conflict. Hence, they will be expected to grow out more or less normally to the surface at which they emerge. Those of early origin will then be found near the foot of the normal drawn from the crystal seed to the growing face, and therefore usually somewhere near the centre of the face, at least in regular crystals. Dislocations of later origin may be anywhere. The observations quoted imply that in these (incidentally, small) crystals the dislocations are few and the probability of fresh generation of dislocations small.

Secondly, Dr. Bunn says "the screw dislocation has been introduced because it is self-perpetuating." This must be corrected. The dislocation was "introduced" from the theory of continuum elasticity into the theory of real crystalline solids about 15 years ago to account for their plastic deformation, and about 10 years ago as the element of misfit into which various derangements of a crystal (such as "mosaic boundaries") can be resolved. Each dislocation is characterized by a "Burgers vector," belonging to a limited family of crystallographic vectors. The idealized "screw dislocation" is that in which the dislocation line lies parallel to its Burgers vector; but if this vector is not parallel to the crystal face at which the dislocation line terminates, the dislocation partakes of screw character, and provides a self-perpetuating terrace on the crystal face. If dislocations are present, it is only in certain special arrangements, for the maintenance of which there is no discoverable reason, that they could fail to possess screw terminations.

Of course, dislocation theory will be in a more satisfactory state when a complete account of their genesis has been given. But I did not omit to discuss the matter in my paper.

Humphreys-Owen raises a very significant point: that the growth-resistance either at a crystal corner, or of a whole face which has ceased growing, is remarkably high. By itself this could mean either that the dislocations were very close together in these areas or that they were absent. It is very much easier to understand the erratic nature of the growth of these small crystals on the latter assumption, which is also consistent with the evidence that the growing point is often near the centre of the face. In fact, when the growing point is not near the face centre of a small crystal, there is a strong likelihood of the production of a "hopper crystal." For then the diffusion field corresponding to uniform

deposition will result in a smaller supersaturation at the face centre than that at the growing point. A certain reduction, by a factor of the order 100, can be compensated by the growth fronts crowding together in the region of low supersaturation, but this compensation is definitely limited, whereas the fall in supersaturation which would accompany uniform deposition might even be to a negative value. In such cases deposition cannot be uniform but will be less near the centre of the face. The result will be a hopper crystal. This conclusion applies to small crystals, or those grown in still media, or grown fast. With larger crystals or good stirring the variation in concentration across a face will be much smaller; on the other hand, when the growth rate is fast the amount of variation in supersaturation which can be compensated by a change in the spacing between step-lines is smaller.

Perhaps I should summarize: the dislocation structure of a crystal has very little effect on its rate of growth (provided it permits it to grow at all) if the supersaturation is the same all over the crystal. The latter is not the case with small crystals, or crystals in a still medium. The dislocation density probably varies greatly, from substance to substance and with the conditions of crystal growth and treatment, but we can learn very little about it from simple measurements of growth rate, or even from the shape and surface topography in growth under usual conditions: it might be anything from 1 per crystal face to  $10^{10}$  or more per square centimetre. But there is evidence in the experiments of Bunn, and of Humphreys-Owen, that it is indeed much nearer to 1 in some of the small crystals they have studied. In that case, with a variation of concentration across the crystal face, which must exist when diffusion rules, the growth rate can be determined by the location of dislocations in the face.

**Dr. U. R. Evans** (*Cambridge*) (*communicated*): Three important growth forms (dendritic, concentric and allotriomorphic), mentioned by several speakers, deserve closer consideration.

**DENDRITIC FORMS.**—Mott<sup>15</sup> attributes dendritic growth to the circumstance that the tip of an advancing needle provides a spot favourable for the replenishment of material or for the dissipation of heat. This widely held view that growth is favoured by a sharp point seems at first sight to conflict with the demonstration of Bunn and Emmett<sup>16</sup> that, under certain circumstances, deposition occurs preferentially at the centres of the faces—that is, at a maximum distance from the sharpest points on the crystal; it is even suggested that the replenishment of material at face centres proceeds more readily than at crystal corners.

The apparent discrepancy may be connected with the fact that, as Bunn himself points out, his theory neglects the growth of the crystal; this assumption, which he describes as unrealistic, is doubtless permissible for many purposes, but in dendritic growth the movement of the tip of the dendrite is probably an essential feature of the mechanism. The impoverishment of a solution, which must always be expected during the advance of a flat face, may be avoided when a fine needle is pushing out into ever fresh regions. Such a mechanism would lead, however, to forms possessing excessive surface energy, and there will often be a tendency for the freshly deposited atoms or ions to re-arrange themselves, possibly by surface diffusion, so that the dendritic form is not developed. As to whether surface-rich or surface-poor forms are observed depends on the relative rates of two processes: (1) the deposition of atoms and (2) their re-arrangement. The second change will be influenced by the specific surface energy and the surface mobility of the particles.

Certain simple experiments<sup>17</sup> on the production of two-dimensional lead trees by pressing the edge of a vertical zinc strip on a filter paper soaked in a solution containing lead acetate may serve to illustrate the principles involved. Filaments of lead quickly push outwards along the paper, and soon the deposition of lead at the growing tips is proceeding nearly a centimetre from the place where zinc is being dissolved. The need for this "action at a distance" becomes clearer on applying a sulphide indicator; it is found that the lead salt has become almost

<sup>15</sup> Mott, *This Discussion*.

<sup>16</sup> Bunn and Emmett, *This Discussion*.

<sup>17</sup> Evans, *Chem. and Ind.*, 1925, 812.

exhausted near the metallic zinc. The lead "plants," unable to find nutriment at home, push out into the world to obtain it. Of course, given time, lead salts would diffuse towards the zinc. But diffusion under a concentration gradient is generally a slower process than ionic migration under a potential gradient; both are due to the same type of movement, but it is random in the first case and "directed" in the second. Whether the flow of electric current along the lead filaments has ever been demonstrated may be doubted; but, in the analogous case of corrosion of zinc by a sodium salt solution and oxygen, the current has been measured<sup>18</sup> and found strong enough to account for the observed corrosion rate.

Clearly, dendritic growth can occur without a flow of current; but it is particularly likely to be met with in the cathodic deposition of a heavy metal where the volume occupied by the metal after deposition is usually much smaller than the volume of solution containing the requisite number of ions, so that, in the absence of stirring, deposition in layers soon becomes impossible if the current, provided from an external battery, exceeds a certain value; thus "treering" has become one of the electrodepositor's nightmares.

CONCENTRIC GROWTH (IN CIRCLES AND SPHERES).—Patterson<sup>19</sup> describes the spreading of rust over a pure iron surface as proceeding in a concentric manner, although on steel mossy growths, recalling dendritic forms, are commoner, as shown also by Vernon.<sup>20</sup>

Spherical growth would, presumably, be the normal form of solid accretion in a universe in which surface energy was independent of direction. Even in our complicated world, it is the normal form of liquid accretion. Matter undergoing a phase change may be regarded as momentarily liquid, and it is tempting thus to seek an interpretation of the pyramid-growth spreading from face centres, as observed by Bunn and Emmett,<sup>16</sup> since this geometrically represents an attempt to attain spherical form. One at least of those who saw the I.C.I. film was impressed by the resemblance of some of the phenomena to liquid flowing out of a hole. But such resemblances are often misleading, and it is best not to press the analogy too far.

The laws of expanding circles and spheres, which are important in connection with surface oxidation and annealing changes, have been developed by a simple method described elsewhere<sup>21</sup>; in cases where other, more tedious, methods are available, the results are in agreement. When films spread over a surface from pre-existing nuclei (sporadically distributed) which start to operate at zero time (no other nuclei appearing thereafter), the fraction of the surface remaining uncovered after time  $t$  will be  $e^{-kt^2}$ ; if nuclei are absent at the outset, but appear (sporadically in time as well as in space) on the ever-diminishing area available, the fraction will be  $e^{-kt^3}$ . For expanding spheres, the corresponding expressions will be  $e^{-kt^3}$  and  $e^{-kt^4}$  respectively. Naturally  $k$  has a different meaning (and different dimensions) in the four cases, as shown in Table I, which also includes the final grain-size obtained when the phase change is complete. Where growth has occurred solely from pre-existing nuclei, the grain size must clearly be independent of the crystallization velocity, being the reciprocal of the nucleus number. However, where there are no pre-existing nuclei, it is proportional to the appropriate power of the ratio of crystallization velocity to nucleation rate; the numerical coefficient arises out of a gamma function. The power ( $2/3$  and  $3/4$  in the two cases) deserves notice, since it has been stated that the grain size is given by the first power of that ratio—which is surely impossible, since the expression would then have the wrong dimensions.

The distinction between the expressions for spreading in the presence and absence of pre-existing nuclei may come to be helpful in distinguishing between the two cases. In some types of phase change, the points where the change originates are apparently not crystal germs, but seem to be points of *atomic disarray* where the energy of inception is lower than elsewhere.

<sup>18</sup> Agar, quoted by Evans, *J. Iron Steel Inst.*, 1940, **141**, 220 P; also Noordhof and Evans (unpublished work).

<sup>19</sup> Patterson, *J. Soc. Chem. Ind.*, 1930, **49**, 206 T.

<sup>20</sup> Vernon, *Trans. Faraday Soc.*, 1924, **19**, 887 (Fig. 21).

<sup>21</sup> Evans, *Trans. Faraday Soc.*, 1945, **41**, 365.

**ALLOTRIMORPHIC FORMS.**—A polycrystalline aggregate formed from a melt in which spherical crystals spread at uniform rate from pre-existing nuclei should consist of grains separated by plane boundaries; if pre-existing nuclei are absent the boundaries will be curved, whilst, if furthermore the outgrowth is dendritic, the boundaries will be irregular and interlocked—as is often observed. On annealing, the boundaries will tend to straighten—thus diminishing the interfacial energy of the system.

TABLE I

**SYMBOLS.**— $v$  represents the radial velocity of crystallization,  $w$  the two-dimensional nucleus number defined by the statement that the chance of finding a nucleus in area element  $da$  is  $w da$ ,  $\Omega$  the two-dimensional nucleation rate defined by the statement that the chance of a nucleus appearing in area element  $da$  and time-element  $\delta t$  is  $\Omega da \delta t$ , whilst  $w'$  and  $\Omega'$  are the corresponding three-dimensional quantities.

Conditions	Value of $k$	Final Grain Size
Two-dimensional: pre-existing nuclei ..	$\pi w v^2$	$1/w$
Two-dimensional: no pre-existing nuclei ..	$\pi \Omega v^2/3$	$1.137(v/\Omega)^{2/3}$
Three-dimensional: pre-existing nuclei ..	$4\pi w' v^3/3$	$1/w'$
Three-dimensional: no pre-existing nuclei..	$\pi \Omega' v^3/3$	$1.117(v/\Omega')^{3/4}$

An early examination<sup>22</sup> of Carpenter and Elam's studies of boundary migration<sup>23</sup> showed that, out of 53 cases, the *apparently* concave grain invaded the *apparently* convex grain in 25 cases, whilst in eight cases the reverse movement was noted; the other 20 cases were doubtful. The existence of eight apparent exceptions to the straightening rule ("concave invades convex") is not surprising, since the curvature was judged from sections and there was no information about the curvature at right-angles to the paper. If the two curvatures are *uncorrelated*, one would expect that the rule would break down once in four times. However, the numbers were felt to be too small for significant conclusions, and plans were made for an extensive research by Cook and the writer which should have provided data for statistical analysis and settled numerous outstanding questions. Preliminary data were published,<sup>24</sup> but, owing to Dr. Cook's departure from Cambridge to take up a post elsewhere, the main research has never been carried out.

The writer wishes to thank Dr. Bunn, Dr. Wooster and Dr. Agar for helpful discussion.

<sup>22</sup> Evans, *J. Inst. Metals*, 1922, **27**, 140.

<sup>23</sup> Carpenter and Elam, *J. Inst. Metals*, 1920, **24**, 104 (Fig. 1 to 24).

<sup>24</sup> Cook and Evans, *Trans. Amer. Inst. Min. Met. Eng.*, 1924, **71**, 627.

## II. NUCLEATION AND NORMAL GROWTH

### Introductory Paper

BY W. J. DUNNING

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The simplest case of crystal nucleation is the separation of two<sup>1</sup> solid solution phases from a single phase on cooling to a point below the critical mixing temperature  $T_c$ , and it is natural<sup>1</sup> to apply Einstein's theory of

<sup>1</sup> Berkeley, *Phil Mag.*, 1912, **254**.



would be considered nuclei of the new phase. Under these conditions the probability of nucleation would be given by

$$W \propto e^{-\frac{1}{2} \left\{ \frac{d^2 A}{dN^2} \right\} \cdot \frac{\Delta N^2}{kT}} \propto e^{-\frac{1}{2} \left( \frac{d \log \alpha}{dN} \right) \frac{\Delta N^2}{kT}} \quad (4)$$

where  $\alpha$  is the activity.

Wictorin <sup>4</sup> has carried out measurements of the rate of separation of the two phases from a gold-platinum alloy during annealing at a temperature just below  $T_c$ . His results were shown by Borelius <sup>5</sup> to be consistent with the view that the rate of nucleation is governed by the magnitude of the curvature of the macroscopic  $A/N$  relation.

Eqn. (4) will not be valid at temperatures far below  $T_c$ , and where a solid precipitates from a liquid phase it is very doubtful if, even in principle, the two branches of the  $A/N$  curve (Fig. 2) can be considered as continuous. In Fig. 2 branch *a* refers to the solution and branch *b* to the almost pure solid. The process of nucleation is no longer visualized as the progressive enrichment of solute in a small volume. Even if the small volume by fluctuations became almost pure solute, we should still be faced with the problem of the discontinuous change of liquid into solid solute. The fluctuations which lead to the separation of the solid solute are therefore conceived as partaking of crystalline properties from the start. The fluctuations begin as two molecules, some attaching a third, and then a fourth molecule and so on, into lattices of localized molecules, until minute structures are formed of the same crystalline nature as the solid solute. The probability of formation of these crystalline fluctuations is considered to be given by eqn. (2) in which  $\Delta A$  is the free energy of formation of this nucleus. On these assumptions this is found to be of the form,

$$\Delta A = \frac{1}{2} \sigma F, \quad (5)$$

where  $\sigma$  is the interfacial free energy of the crystal in contact with the supersaturated solution, and  $F$  is the area of the surfaces of the crystal.

Again these fluctuations have an overall tendency to redisperse until they reach a critical size ("radius" =  $r_k$ ) given by an equation analogous to the Gibbs-Thomson equation,

$$RT \log \frac{c_1}{c_{10}} = \frac{2\sigma M}{r_k \cdot \delta} \quad (6)$$

where  $c_1$  and  $c_{10}$  are the concentrations of the supersaturated and saturated solutions,  $M$  is the molecular weight and  $\delta$  the density of the crystal. At this critical size, the tendency to redisperse is equal to the tendency to grow. Above this critical size there is an overall tendency to grow. Fluctuations of this critical size are "nuclei." Using eqn. (2), (5) and (6) the rate of nucleation is given by

$$J = e^{-\frac{16}{3} \frac{N \pi M^2 \sigma^2}{R^2 \delta^2 T^2 \left( \log \frac{c_1}{c_{10}} \right)^2}} \quad (7)$$

( $N$  is Avogadro's number). This theory of Volmer and Weber <sup>6</sup> was improved by Stranski and Kaischew <sup>7</sup> and later by Becker and Döring.<sup>8</sup>

These latter considered the detailed mechanism by which the fluctuation grows towards and passes the critical size. Considering the separation of a cubic homopolar crystal, and the crystalline fluctuation at a stage in its growth, the incident molecules are laid down one at a time on the surface.

<sup>4</sup> Wictorin, *Ann. Physik*, 1938, **33**, 509.

<sup>5</sup> Borelius, *Ann. Physik*, 1938, **33**, 517.

<sup>6</sup> Volmer and Weber, *Z. physik. Chem.*, 1926, **119**, 277.

<sup>7</sup> Stranski and Kaischew, *Z. physik. Chem. B*, 1934, **26**, 317.

<sup>8</sup> Becker and Döring, *Ann. Physik*, 1935, **24**, 719.

This kinetic treatment considers three stages of the growth, the formation of linear chains, the formation of two-dimensional islands on completed surfaces, and the formation of three-dimensional lattices. Becker and Döring assume no restrictions on the possibilities of attachment of the units, but they are able to show that the free energy of the system is smallest when the islands are square and the lattices are cubic. There is a critical cube size and a critical square size corresponding to three- and two-dimensional nuclei respectively. Equations are derived for the linear rate of growth and for the rate of homogeneous nucleation.\*

A comprehensive experimental check on the theory would require to show that the rates of nucleation and growth have the correct dependence upon the supersaturation, the temperature and the surface and edge free energies, and further that the pre-exponential factor is correct. A partial check has been carried out by Amsler.<sup>9</sup> He showed that the effect of supersaturation upon the induction period of nucleation (taken as inversely proportional to  $J$ ) has a form related to that of Fig. 3. Further work with the same object in view is reported in this Discussion by Van Hook and

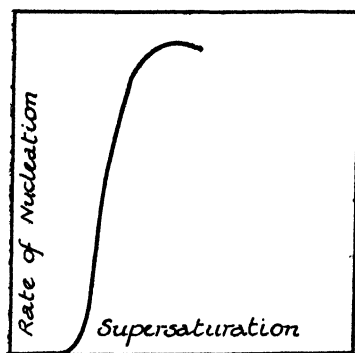


FIG. 3.

Bruno, and by Bransom, Dunning and Millard. The general impression one gets is that the experiments broadly confirm the theory, though from the latter authors' results there appears a large discrepancy in the value of the pre-exponential factor. This may have a bearing on the fundamental nature of the fluctuations. Possibly at some stage in the formation of the nucleus the fluctuation changes from non-localized to localized molecules. Information on this is most likely to be gained by studying highly supersaturated solutions where the critical size is small. Possible techniques for such experiments are considered by Bransom and Dunning, though only results for low supersaturations

are reported. A paper by Davies and Jones describes experiments in which the nucleation of supersaturated solutions of silver chloride is followed by means of conductivity measurements. They find that the metastable limit of the solubility product varies with the ionic ratio of  $\text{Ag}^+$  to  $\text{Cl}^-$  ions. This observation requires to be accounted for by quantitative theory; presumably the views of Krut and Verwey<sup>10</sup> on the structure of silver halide sols would be of interest here.

The theory of Becker and Döring was derived for nucleation from the vapour phase. Its application to solutions must be restricted to some extent by diffusion processes. Since many experimental observations on nucleation require the nuclei to grow or be developed to observable or measurable size, its principal effect will be on this growth process. Neumann<sup>11</sup> has considered this matter and it would appear that nucleation studies in unstirred tubes<sup>12</sup> may give results in which diffusion effects would mask any possible correspondence with nucleation theory. Van Hook, in this Discussion, also reports the effect of stirring upon nucleation.

\* Amsler, *Acta Physic. Helv.*, 1942, **15**, 699.

<sup>10</sup> Krut and Verwey, *Symposium on Hydrophobic Colloids* (Amsterdam, 1938).

<sup>11</sup> Neumann, in Volmer's *Kinetik der Phasenbildung*, p. 209, et seq.

<sup>12</sup> Dehlinger and Wertz, *Ann. Physik*, 1939, **36**, 226.

\* See Bransom, Dunning and Millard, this Discussion, eqn. (15) and (19).

It is, however, in the growth of crystals that diffusion may play its significant role. In the papers on the growth of crystals in Section II, it is necessary to distinguish broadly between those observations in which the growth of isolated crystals is studied and those in which the average growth of a large number of crystals is studied, and between those in which the solution is stirred and those in which the solution is stagnant. In the latter it appears that diffusion may play a dominant role. Again it must not be forgotten that the theory is a statistical one.

In the work of Bransom, Dunning and Millard, average growths in stirred solutions were measured and the interesting point which appears is that, contrary to the theory of Becker and Döring, the linear rate of growth is independent of the size of the crystal. This may mean that either the crystals have a mosaic or lineage structure, each of which requires two-dimensional nucleation and that growth planes are halted at the discontinuities, or that there is a constant surface density of dislocations, the nature of which is discussed by Frank.

In contrast to this work on non-polar crystals, which is broadly in accordance with theory, the observations of Bunn, Everett, Berg and Humphreys-Owen are very difficult to reconcile with theory. No connection is found between the rate of growth and the supersaturation at the surface. The growth rate of a single face changes for no apparent reason and similar faces have widely different rates. Further growth starts at the centre of a face and spreads outward, apparently in thick layers (except in the case of non-polar crystals). Bunn is of the opinion that the effects are traceable to the characteristic distribution of the diffusion gradient. This is attributed to a compromise between a tendency to radial diffusion and a tendency for the crystal to remain polygonal. The result is that the gradients are not quite radial and the surfaces of the crystal are not quite flat.

Suggestions have been made that perfect crystals do not grow under mild conditions of supersaturation, and that growth on natural crystals proceeds by a mechanism involving dislocations. Fordham contributes some measurements from which it appears that distorted ammonium nitrate crystals grow more quickly than undistorted.

The question of foreign nucleation as opposed to homogeneous nucleation is a subject which properly belongs to Section III of this Discussion. It is sufficient to call attention here to the work of Tschermak-Seysenegg,<sup>13</sup> who found that nucleation was very specific. For example, sodium acetate trihydrate solutions could not be nucleated with the monohydrate nor with the potassium salt. Incidentally his work on the electrical effects which accompany crystallization is of considerable interest.

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<sup>13</sup> Tschermak-Seysenegg, *Z. Krist.*, 1939, **101**, 230.

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## KINETICS OF CRYSTALLIZATION IN SOLUTION

### Part I

BY S. H. BRANSOM, W. J. DUNNING AND B. MILLARD

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The formation of a new phase from a mother phase can be considered as involving two processes, the formation of three-dimensional nuclei and



the growth of these critical nuclei to macroscopic dimensions. The experimental study of the first process particularly is rendered difficult by the fact that in early stages of the formation the two processes take place simultaneously. The nuclei themselves are difficult to observe and count, and it appears necessary to "develop" them to an observable size by growth. Such experiments must therefore be designed so that the added complication brought in by the development process can be evaluated and taken into account. Such experimental techniques are, we believe, described in Part I and II of our investigations.

In this work, the use of salts as the precipitating phase has been avoided, since it was considered that there might be present complications of electrostatic origin, arising from diffusion potentials and differential adsorption. In this initial exploration we have therefore restricted ourselves to cyclonite. This material has the advantages of being non-ionic; it is soluble in a number of solvents but is relatively insoluble in water; it is stable and does not decompose in solution; it crystallizes from many solvents in isometric habits which approximate to spheres; it has a high melting point and its crystals are hard and do not easily suffer attrition.

**Continuous Crystallization.**—If a solid (cyclonite) can be precipitated from its solutions in a solvent (concentrated nitric acid) by the addition of another miscible solvent (water), the process of crystallization can be studied in the following manner. The solution and the precipitating liquid are fed continuously and at a steady rate into a vessel. This vessel is provided with an outflow through which, once the vessel is filled, the contents pass out at a steady rate. With efficient stirring, the composition of the outflow is the same as the contents of the vessel. With time the system approaches a steady state in which nuclei are forming at a constant rate, crystals are growing at a constant rate (since the degree of supersaturation is constant) and a constant proportion of the entire contents of the vessel is passing through the outflow in unit time.

Let the volume of the contents of the vessel be  $V$ , and the rate of efflux  $v$  per sec. in same units of volume, this is equal to the sum of the rates of influx assuming that there is no volume change on mixing and crystallization. Let  $n_0$  be the number of nuclei which form per unit volume in a short interval of time  $dt_0$  at time  $t_0$ . At some later time  $t_1$  some of these nuclei born at time  $t_0$  have been lost through the outflow and those which have remained in the vessel ( $n$ ) have grown in size under the influence of the steady supersaturation. At the time  $t_1$  the rate of loss of these crystals is

$$-\frac{dn}{dt_1} = n \frac{v}{V}, \quad . \quad . \quad . \quad . \quad . \quad (1)$$

or putting  $t_1 - t_0 = \theta$ , the age of the crystals,  $d\theta = dt_1$ , and

$$\frac{dn}{d\theta} = n \frac{v}{V}, \quad . \quad . \quad . \quad . \quad . \quad (2)$$

hence

$$n = n_0 e^{-v\theta/V} \quad . \quad . \quad . \quad . \quad . \quad (3)$$

If the linear rate of growth of the crystals depends only on the supersaturation  $S$  we can put

$$dr/d\theta = f(S) \quad . \quad . \quad . \quad . \quad . \quad (4)$$

Hence

$$r = f(S)\theta, \quad . \quad . \quad . \quad . \quad . \quad (5)$$

where  $r$  is an average dimension of the crystal, which for isometric crystals may be considered as the "radius." Substituting for  $\theta$  from (5) into (3) we get

$$n = n_0 e^{-vr/Vf(S)} \quad . \quad . \quad . \quad . \quad . \quad (6)$$

For those crystals which were born at a time  $t_0$ , there is therefore a relation (6) between  $n$  the number which remain in the vessel and their size. In the stationary state the size distribution of the crystal population in the vessel is stationary and does not change with time. Using relations (6) and (4) we have for this distribution,

$$n(r) = \frac{n_0}{f(S)} e^{-vr/Vf(S)}, \quad . \quad . \quad . \quad . \quad (7)$$

where  $n(r)dr$  is the number of crystals per unit volume of suspension which have linear dimensions between  $r$  and  $r + dr$ . Thus, if the contents of the vessel are analyzed and the number distribution determined, we have

$$\log n(r) = \log \frac{n_0}{f(S)} - \frac{vr}{Vf(S)}. \quad . \quad . \quad . \quad . \quad (8)$$

By plotting  $\log n(r)$  against  $r$  we can obtain  $v/Vf(S)$  and hence  $f(S)$  from the slope and then  $n_0$  from the intercept at  $r = 0$ .

### Experimental

Fig. 1 shows the arrangement of the apparatus. The crystallizing vessel A was cylindrical with a hemispherical bottom and had an outflow tube set in the side at a fairly steep angle. Vessel and outflow were jacketed and water was circulated through the jacket from a thermostat. The solution and diluent flowed from two jacketed Mariotte bottles B, B at constant rates which could be adjusted by altering the levels of the air inlets  $b, b$ . The jets of the Mariotte bottles passed into the vessel through the closely fitting lid C, through which also passed a thermometer. This lid carried two semi-parabolic baffles on either side of a propeller-like stirrer. By consideration of such factors as the depth and diameter of the vessel A, the shapes and positions of the propeller and baffles, speed and direction of thrust of the stirrer, it is possible to minimize any tendency of the crystals to sediment and to feed in the reactants so that the local supersaturations do not deviate much from the average throughout the vessel. All these items were mounted on a single vertical rod pivoted at its lower end, and clamped at its upper end. The rod could be released and the apparatus tipped forward to spill the contents of A into a jacketed filter D beneath which was a weighing bottle E to catch the filtrate.

Before beginning the experiment water was circulated round the vessels, the Mariotte bottles adjusted to give the required rates of flow and the stirrer started in the empty vessel. Since the stirrer created a vortex any change in the speed of the stirrer during the experiment is to be avoided, otherwise the effective volume of the vessel changes. Then the influx of the solution and the diluent were started simultaneously and the time to fill the vessel to the point of outflowing is noted. The rate of outflow was noted several times during the course of the experiment to check the constancy of the rates of inflow. The experiment was then allowed to run until most of the contents of B, B had been delivered, hence the amount of fluid passing through the vessel A was about thirty to forty times the volume. To analyze the contents of the vessel the following procedure was adopted. Firstly, a running sample of the outflow was collected in order to determine later the overall composition in terms of the three components; these figures were checked against the known composition of the reactants and their rates of influx. After noting the temperature, the vertical bar was released to spill the contents of the reaction vessel into the filter D and the crystals rapidly filtered. The filtrate was supersaturated and was later weighed and analyzed and the results used for calculation. The crystals were washed free from mother liquor, and after drying and weighing were ready for particle size analysis. Immediately the sample had been tipped out, the inlet feeds were stopped and the remaining contents of the vessel allowed to stir for 30 min. The supersaturation in the vessel was thereby reduced to saturation, the temperature was noted since there is a slight fall in temperature when there is no heat of mixing being produced and the contents of the vessel again tipped through another jacketed filter and the filtrate

collected. An analysis of this filtrate gave the solubility in the actual solvent; this figure could be adjusted to the running temperature by means of the known temperature coefficient of solubility. This adjustment was usually small.

The observations recorded were: (1) composition of the liquid phase; (2) amount of solid in suspension; (3) particle size distribution; (4) degree of supersaturation; (5) temperature; (6) effective volume of the vessel; (7) rate of influx and efflux ( $v$ ). From these figures a number of cross-checks could be made to ensure that the necessary theoretical conditions were in effect.

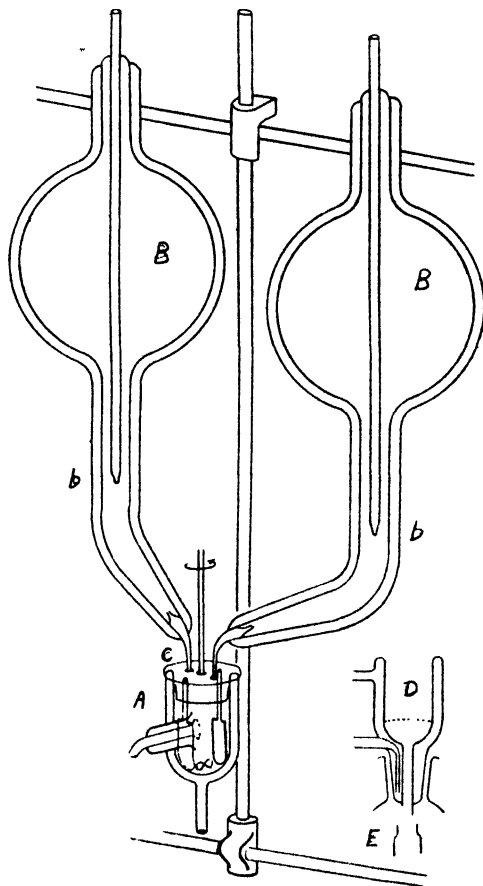


FIG. 1.

The particle size distribution was obtained by means of a photoelectric sedimentometer, a description of which has appeared elsewhere.\* By means of this instrument either  $n(r)$  or  $W(r)$ , the weight of particles which have radii between  $r$  and  $r + dr$ , can be determined. In the present case,  $W(r)$  was measured and this is related to  $n(r)$  by

$$W(r) = \gamma n(r) r^3 d, \quad (9)$$

where  $d$  is the density and  $\gamma$  is a shape factor,  $\gamma$  is equal to  $4\pi/3$  if the particles are spheres. On this basis eqn. (9) becomes

$$W(r) = \frac{4\pi d r^3 n_0}{f(S)} \cdot e^{-vr/V(S)} \quad (10)$$

\* Bransom and Dunning, *J. Soc. Chem. Ind.*, 1949, **68**, 80.

Fig. 2 *a* shows this function and Fig. 2 *b* a typical result obtained in the present experiments. In Fig. 3 the plot of  $\log n(r)$  obtained from Curve 2 *b* is shown; this is derived by using eqn. (9). Eqn. (8) shows that if the theoretical conditions are fulfilled  $\log n(r)$  should be a straight line when plotted against  $r$ , and it is

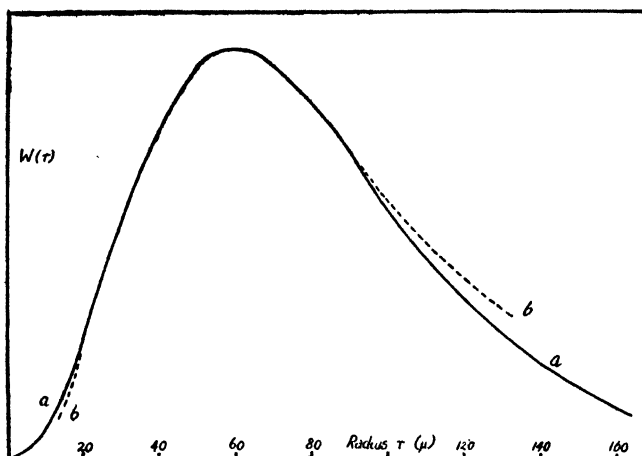


FIG. 2.

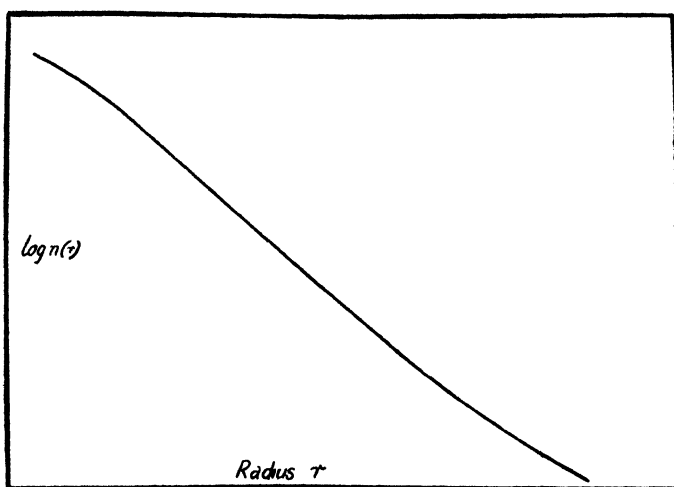


FIG. 3.

seen that the deviations are small. The slope of this curve gives  $f(S)$  according to (8). The position of the maxima on these  $W(r)$  against  $r$  curves is easily shown to be

$$r_{\max.} = \frac{3Vf(S)}{v}, \quad . \quad . \quad . \quad . \quad . \quad (11)$$

which allows a check on the values of  $f(S)$  derived from the slope of the  $\log n(r)$  against  $r$  plot.

The intercept of the  $\log n(r)$  against  $r$  line with the ordinate for  $r = 0$  gives a value of  $n_0$ . Since, however, the results of the photoelectric sedimentometer are not easily interpretable for crystals smaller than about  $r = 10 \mu$ , an extrapolation has to be made here, but the relative values of  $n_0$  and the order of magnitude should be reliable, unless large and varying slopes occur between

$r = 10 \mu$  and  $r = r_h$  the radius of the nucleus. Another value of  $n_0$  can be obtained from  $r_{\max}$  by means of the relation,

$$n_0 = \frac{\gamma}{8\pi Vd} \left( \frac{3}{r_{\max}} \right)^3, \quad . \quad . \quad . \quad . \quad (12)$$

where  $\gamma$  is the yield of crystals per unit of time from the outflow, and  $n_0$  is the number of nuclei formed per unit of time per unit volume. Eqn. (12) serves as a check on the previous value obtained for  $n_0$ .

### Results

Table I gives the results obtained for a series of experiments at about  $67^\circ \text{C}$ ; groups of these experiments were carried out for times of passage ( $V/v$ ) in the regions of 2.5, 6 and 14 min. The third column gives the percentage of nitric acid in the mother liquor of the crystallizing vessel A, and these values were reasonably constant at  $54\% \pm 1$ . Definite trends are noticeable between the factors  $V/v$ ,  $r_{\max}$ , the rate of linear growth  $f(S)$  and the rate of nucleation.

TABLE I

Date of Expt.	Temp. ( $^\circ \text{C}$ )	Mother Liquor Comp. %	Time of passage (min.)	$r_{\max}$ .	$f(S)$ $\mu/\text{min.}$	Supersaturation	$n_0/\text{ml. min.}$
23.2.42	66.5	54.1	2.40	58	8.6	.016	$8.4 \times 10^4$
27.2.42	68.5	54.4	2.25	52	7.7	.047	$9.3 \times 10^4$
16.3.42	67.5	56.0	2.60	54	7.6	.018	$7.0 \times 10^4$
26.5.42	66.7	53.1	3.0	52	6.4	.066	$7.2 \times 10^4$
28.5.42	68.8	53.0	2.8	57	7.9	.039	$6.2 \times 10^4$
3.6.42	67.8	53.7	2.9	57	6.9	.030	$7.4 \times 10^4$
Average			2.7	56	7.6	.043	$7.3 \times 10^4$
5.6.42	66.4	54.7	5.7	84	5.6	.044	$1.4 \times 10^4$
8.6.42	65.6	53.0	6.1	80	4.0	.020	$1.7 \times 10^4$
9.6.42	65.0	54.6	6.1	70	3.5	.020	$2.6 \times 10^4$
Average			6.0	78	4.3	.028	$1.9 \times 10^4$
17.6.42	66.0	52.6	13.5	130	2.6	.006	$0.28 \times 10^4$
18.6.42	66.0	53.9	14.5	130	2.9	.010	$0.20 \times 10^4$
2.6.42	66.3	53.6	14.5	105	2.1	.030	$0.30 \times 10^4$
Average			14.0	120	2.5	.015	$0.26 \times 10^4$

The least satisfactory are the measurements of the supersaturation which exhibit a wide scatter. Nevertheless, comparison of averages in columns 6 and 7 suggests that the rate of linear growth is a linear function of the supersaturation over the limited range studied, i.e.,

$$dr = kSd\theta \quad . \quad . \quad . \quad . \quad (13)$$

or

$$r = kS\theta. \quad . \quad . \quad . \quad . \quad (13a)$$

The trend of  $n_0$  with the supersaturation (eighth and seventh columns) is such that  $n_0$  is a function of a higher power of the supersaturation than the first. An empirical relation would be

$$n_0 = qS^3 \quad . \quad . \quad . \quad . \quad (14)$$

### Discussion

Whilst the above results are limited in extent and precision they exhibit trends which are worth examination. Volmer<sup>1</sup> has modified the theory of Becker and Döring<sup>2</sup> to obtain equations for the linear rate of growth of a

<sup>1</sup> Volmer, *Kinetik der Phasenbildung* (Steinkopff, Dresden and Leipzig, 1939).

<sup>2</sup> Becker and Döring, *Ann. Physik*, 1935, **24** (5), 719.

crystal and the rate of homogeneous nucleation. Volmer gives for  $g$  (the linear rate of growth of a crystal in cm./sec.) the expression,

$$g = w_1 F \delta \kappa \frac{\mu_1 - \mu_{1\infty}}{kT} e^{-A'/kT} e^{-A''/kT}, \quad (15)$$

where  $\mu_1 - \mu_{1\infty} = kT \log \frac{\alpha_1}{\alpha_{10}} = kT \log \frac{c_1}{c_{10}} \sim$ ,

$$kT \cdot \frac{c_1 - c_{10}}{c_{10}} = kTS. \quad (16)$$

$\mu_1$ ,  $\alpha_1$ ,  $c_1$  are the chemical potential, the activity and the concentration of the solute in the supersaturated solution and  $\mu_{1\infty}$ ,  $\alpha_{10}$ ,  $c_{10}$  the corresponding quantities in the saturated solution, and  $S$  is the supersaturation.  $A'$  and  $A''$  are the activation energies for the formation of "one-dimensional" and "two-dimensional" nuclei on the surface of the crystal;  $\delta$  is the distance between the crystal planes normal to the direction of growth, and  $\kappa$  is the length of side of the square two-dimensional nucleus. Since this nucleus is in quasi-equilibrium with the supersaturated solution, an equation analogous to the Gibbs-Thomson equation can be written

$$\kappa = \frac{\rho \delta}{\mu_1 - \mu_{1\infty}}, \quad (17)$$

where  $\rho$  is the edge free energy. The factor  $w_1 F$  represents the number of solute molecules which encounter the crystal surface of area  $F$ . From this it would appear that the rate of linear growth should be proportional to the radius or to the square of the radius of the crystal.<sup>3</sup> If this were so our analysis of the stationary state in the reaction vessel (eqn. (1)-(7)) would not give a Poisson distribution for  $n(r)$  and since such a distribution is obtained experimentally,  $g$  must be independent of  $r$ . Also  $w_1 F$  may be expected to depend upon the first power of the solute concentration and upon the diffusion coefficient of the solute.

With these modifications there results

$$g = \text{const.} \cdot \frac{c_1}{T} \rho f(D) e^{-A'/kT} e^{-A''/kT}, \quad (18)$$

where  $f(D)$  is some function of the diffusion constant  $D$ ,

$$\log g = \log \frac{\rho f(D)}{T} e^{-A'/kT} - \log c_1 - \frac{A''}{kT}. \quad (19)$$

At 340° K and near to it, we will treat the logarithm on the right-hand side as constant since  $A'/kT$  may be expected to be of less significance than  $A''/kT$ , and we have no data concerning the temperature dependence of  $D$ . From <sup>4</sup>,

$$\frac{A''}{kT} = \frac{\omega M \rho^2 N_0}{2d\delta R^2 T^2 S}, \quad (20)$$

where  $\omega$  is a shape factor (taken as  $2\pi$ ). We now have

$$\log g = \log (\text{const.}) - \log c_1 - 4.78 \times 10^{17} \left( \frac{\rho^2}{T^2 S} \right).$$

From this equation for the rate of linear growth, the edge free energy  $\rho$  may be calculated in two ways, from the dependence upon the supersaturation  $S$  at constant temperature and from the dependence upon temperature

<sup>3</sup> Smoluchowski, *Z. physik. Chem.*, 1918, **92**, 129.

<sup>4</sup> Volmer, *Kinetik der Phasenbildung* (Steinkopff, Dresden and Leipzig, 1939), p. 104 and 183.

at constant supersaturation. The first method is more free from objection since at constant temperature  $D$  and the encounter frequency (since the supersaturations are small in these experiments) are constant. The second method is dependent upon the assumption that the temperature coefficients of  $D$  and  $\rho$  are constant and that the encounter frequency  $w_1 F$  merely increases proportionally to the increased solubility.

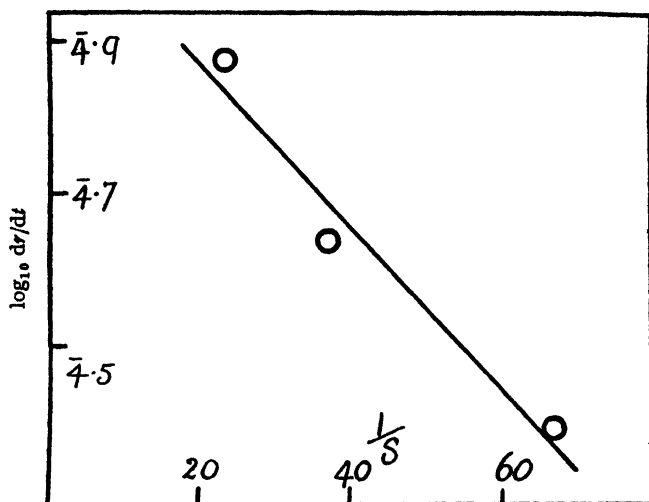


FIG. 4.

For the first method we have at  $T = 340^\circ \text{K}$  and  $c_r$  constant :

$$\log g = \log dr/dt + \text{const.} = \text{const.} - 4.14 \times 10^{12} \frac{\rho^2}{S}.$$

In Fig. 4,  $\log_{10} dr/dt$  is plotted against  $1/S$ , the values being the averages in Table I. A straight line has been drawn between the three points, and from its slope it is found that

$$\rho = 7.4 \times 10^{-8} \text{ erg/cm.}$$

The second method gives for  $T_1 = 340$  and  $T_2 = 349^\circ \text{K}$  with the solubilities  $c_r$  equal to 1.13 and 1.43 g./100 g. acid at  $T_1$  and  $T_2$  respectively,

$$\log \frac{g_2}{g_1} = \log \frac{c_{12}}{c_{11}} - 4.78 \times 10^{17} \frac{\rho^2}{S} \left( \frac{1}{T_2^2} - \frac{1}{T_1^2} \right).$$

Table II gives the rate of growth at  $349^\circ$  and  $S = 0.022$  as  $8.0 \times 10^{-4}$  cm./min. and from Fig. 4 the value at  $S = 0.022$  is interpolated. From these

$$\rho = 22 \times 10^{-8} \text{ erg/cm.}$$

TABLE II

Date of Expt.	Temp. °C	Mother Liquor %	Time of passage (min.)	$r_{\text{max}}$	$f(S)$	Supersaturation	$n_0$
10.3.42	76.1	56.4	2.65	57	8.4	.011	$5.9 \times 10^4$
11.3.42	75.8	54.2	2.75	58	7.8	.030	$5.9 \times 10^4$
12.3.42	75.0	56.2	2.75	55	7.7	.025	$5.9 \times 10^4$
Average			2.70	57	8.0	.022	$5.9 \times 10^4$

In a similar manner the free surface energy  $\sigma$  may be calculated from the results in two ways. The theoretical relation derived by Volmer<sup>5</sup> is

$$J = w_1 Z_1 n_k e^{2\lambda/3kT} e^{-A''/kT} e^{-A'''/kT}, \quad (21)$$

where  $A''$  is again the activation energy for surface nucleation and  $A'''$  the activation energy for homogeneous nucleation. Using Volmer's relations we have

$$n_k = \frac{2A'''}{\mu_1 - \mu_{1\infty}} = \frac{2A'''}{kTS}, \quad (22)$$

and

$$J = w_1 Z_1 \frac{2A'''}{kTS} e^{2\lambda/3kT} e^{-A''/kT} e^{-A'''/kT}, \quad (23)$$

which now bears a close resemblance to Becker and Döring's equation,

$$J = w_1 Z_1 \frac{2A'''}{kT} e^{-(2-1/\kappa)\lambda/3kT} e^{-A''/kT} e^{-A'''/kT} \quad (24)$$

except for the factor involving  $\lambda$  the heat of solution and a term  $(\mu_1 - \mu_{1\infty})^{-1} \sim 1/S$ . For our present purpose we will use an equation without the  $\lambda$  terms:

$$J = w_1 Z_1 \frac{2A'''}{kTS} e^{-A''/kT} e^{-A'''/kT}, \quad (25)$$

with

$$\frac{A'''}{kT} = \frac{16\pi N_0 M^2}{3R^3 d^2} \left( \frac{\sigma^3}{T^3 S^2} \right), \quad (26)$$

we then obtain in the neighbourhood of 340° K,

$$\log J = \text{const.} - 3 \log S - 2.7 \times 10^5 \frac{\sigma^3}{T^3 S^2},$$

in which we have included  $A''/kT$  in the constant term.

The surface free energy  $\sigma$  may be calculated from the dependence at constant temperature of the rate of nucleation  $n_0$  upon the supersaturation  $S$ . At 340° the relation becomes

$$\log J = \log n_0 - \text{const.} = \text{const.} - 3 \log S - 6.9 \times 10^3 \frac{\sigma^3}{S^2}.$$

In Fig. 5,  $\log n_0 + 3 \log S$  has been plotted against  $1/S^2$  and a straight line drawn between the points. From the slope we obtain a value of

$$\sigma^3 = 0.25 \text{ (erg/sq. cm.)}^3.$$

Another evaluation may be obtained from the temperature dependence at constant supersaturation. It must be remembered that the encounter frequency  $w_1 Z_1$  may be expected to depend upon the diffusion coefficient and upon the square of the solute concentration. We shall for the present purpose assume that the diffusion coefficient is independent of the temperature. Thus,

$$\log J = \log n_0 + \text{const.} = \text{const.} + 2 \log c_1 - 2.7 \times 10^5 \frac{\sigma^3}{T^3 S^2}.$$

The value of  $n_0$  at 349° and  $S = 0.022$  is  $5.9 \times 10^4$ , a value of  $n_0$  at 340° K and the same supersaturation can be interpolated with the aid of Fig. 5, and this is  $n_0 = 2.84 \times 10^4$ . Using the values of the solubilities given above we arrive at a value,

$$\sigma^3 = 0.27 \text{ (erg/sq. cm.)}^3.$$

These values give  $\sigma = 0.64$  erg/sq. cm. Too much stress ought not to be laid upon their concordance, considering the assumptions made, especially

<sup>5</sup> Volmer, *Kinetik der Phasenbildung* (Steinkopff, Dresden and Leipzig, 1939), p. 178.



in the latter case and particularly in view of the scatter in the experimental results. The order of magnitude of  $\sigma$  can sometimes be estimated from the heat of solution. The temperature coefficient of the solubility gives a value of  $\lambda = 4.2 \times 10^{-18}$  erg/molecule. Taking the surface area of a molecule as  $68^2$ , this gives  $\sigma \sim 13$  erg/sq. cm. This is about 20 times greater than the derived figure but does not make allowance for surface effects such as interphase potentials which will occur only in the presence of the bulk phase, as distinct from the molecularly dispersed phase, and also of effects such as preferential adsorption of one of the solvents. The difference between the estimated value, which is a surface heat content and the required surface free energy due to the surface entropy factor will probably be a minor part of the discrepancy.

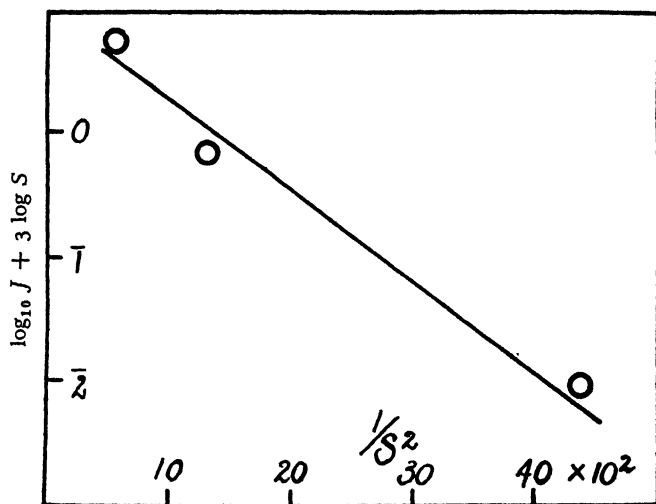


FIG. 5.

If any weight can be given to the degree of concordance between the two values of  $\sigma$  compared to the wider discrepancy between the two values of  $\rho$ , an explanation might lie in the relative importance of diffusion in the encounter processes of the two mechanisms. It seems reasonable that diffusion will play a greater role in the growth of a large crystal where the large surface demands solute from a linear concentration gradient, than in the formation of a nucleus where the small nucleus is at the centre of a radiating concentration gradient.

The following figures give some idea of the orders of magnitude involved. They are calculated for  $T = 340^\circ \text{K}$ ,  $S = 0.022$ ,  $\rho = 7.4 \times 10^{-8}$ , and  $\sigma = 0.64$ . The surface nucleus contains 45 molecules, the three-dimensional nucleus ( $n_k$ ) contains 320 molecules,  $A''/kT = 1$ ,  $A'''/kT = 3.62$ , and  $\lambda/kT = 10$ . The surface nucleus is thus about the size of one side of the three-dimensional nucleus, assuming the latter to be a cube. We included  $A''/kT$  with the constants in our calculations of  $\sigma$ . This is justified if we use the value of  $7.4 \times 10^{-8}$  for  $\rho$  since the variation in this factor is but 10 % of the variation of  $A'''/kT$  with temperature. It is not justified if  $\rho$  is taken as  $22 \times 10^{-8}$ . If the latter figure were used to derive a new value for  $\sigma$  from the temperature coefficient of  $n_0$ , a negative value of  $\sigma$  would be obtained.

The neglect of the term in  $\lambda/kT$  in the calculation of the temperature coefficient seems very serious in view of its order of magnitude. Towards the end of his calculation, Volmer<sup>5</sup> makes the following identifications :

$$\left. \begin{aligned} 4\kappa^2(\epsilon_2 - \epsilon_3) &= A''' \\ \kappa(\epsilon_2 - \epsilon_3) &= A'' \\ 3(\epsilon_2 - \epsilon_3) &= \lambda \end{aligned} \right\} \quad . \quad . \quad . \quad (27)$$

We have already seen that there is a discrepancy between  $\epsilon_2 - \epsilon_3$ , which is the surface energy per atom and the estimated value of the surface energy as calculated from the heat of solution. An alternative method is to calculate  $3(\epsilon_2 - \epsilon_3)$  from our experimental values of  $A'''$  and  $A''$ . We then have  $n_s = (2\kappa)^3 = 320$ , whence  $4\kappa^2 = 46.7$ . The size of the surface nucleus is 45 molecules, from which  $\kappa = 3.4$ . Then

$$\frac{\epsilon_2 - \epsilon_3}{kT} = \frac{3.62}{46.7} = 0.08,$$

and

$$\frac{\epsilon_2 - \epsilon_3}{kT} = \frac{1}{3.4} = 0.29.$$

Volmer's exponent is  $2(\epsilon_2 - \epsilon_3)/kT$  which equals 0.15 or 0.5, and so this exponent may be much smaller than the substituted value of  $2\lambda/kT$  which he used. The contribution of this term to the temperature coefficient of  $n_0$  is then only about 1-2 % of that observed.

The most convenient way of comparing the order of magnitude of the experimental and theoretical rates of nucleation is to compare the values of the factor  $w_1 Z_1$ . Inserting the values obtained for  $S = 0.022$  and  $T = 340^\circ \text{K}$  in Volmer's equation (23) it is found that

$$\log_{10} w_1 Z_1 = 2.2.$$

For crystallization from a supersaturated vapour, Becker and Döring describe  $w_1 Z_1$  as the gas kinetic binary collision frequency. By analogy,  $w_1 Z_1$  in our case should be the binary encounter frequency. Studies of chemical reaction kinetics in solution<sup>6</sup> suggest that the binary collision frequency between solute molecules would be in our experiments of the order  $10^{28}$  per sec. This figure probably includes repetitive collisions and it is likely that in nucleation the encounter frequency would be more appropriate. Considerations based on the work of Smoluchowski,<sup>8</sup> Bradley<sup>7</sup> and Ölander<sup>8</sup> suggest that the encounter frequency is but one or two powers of ten smaller. The resulting figure for  $w_1 Z_1$  of  $10^{26}$  or  $10^{27}$  is very far from the experimental result.

In their calculation Becker and Döring consider only energy terms, and towards the end make the substitutions given in our eqn. (27) and (28), in which total surface energy appears to have been confused with free surface energy. Thus it might appear that entropy terms have been neglected throughout their calculation, and that the large decrease in entropy on crystallization would introduce a factor which would considerably reduce the probability of nucleation. In order to investigate more closely this question, it is convenient to transcribe Becker and Döring's treatment into Eyring's nomenclature.<sup>9</sup>

<sup>6</sup> Moelwyn-Hughes, *Kinetics of Reactions in Solution* (O.U.P.). Hinshelwood, *Kinetics of Chemical Change* (O.U.P.).

<sup>7</sup> Bradley, *J. Chem. Soc.*, 1935, 1910.

<sup>8</sup> Ölander, *Z. physik. Chem.*, 1929, **144**, 118.

<sup>9</sup> Glasstone, Laidler and Eyring, *The Theory of Rate Processes* (McGraw-Hill, New York and London, 1941).

In thus sketching their treatment, we assume that the partial potential of the solute in the supersaturated solution is given by

$$\mu_i = -kT \left( \log \frac{f_i}{x} + 1 \right), \quad . \quad . \quad . \quad (28)$$

where  $x$  is the mole fraction and  $f_i$  the partition function of a solute molecule. For the infinite solid,

$$\mu_{is} = -kT \log f_s \quad . \quad . \quad . \quad (29)$$

where  $f_s$  is the partition function of a molecule in the solid. The rate of deposition of solute molecules onto the surface of a crystal is given by

$$x \omega \frac{kT}{h} \frac{P_{i+1}^{\dagger}}{P_i f_i e}, \quad . \quad . \quad . \quad (30)$$

where  $\omega$  is a weight factor (number of sites available),  $P_i$  is the partition function of the crystal onto which the molecule is depositing and  $P_{i+1}$  is the partition function of the activated state consisting of the crystal and the depositing (or dissolving) molecule. The rate of solution of molecules from the crystal is given by

$$\omega \frac{kT}{h} \frac{P_{i+1}^{\dagger}}{P_{i+1}} \quad . \quad . \quad . \quad (31)$$

At equilibrium between the saturated solution (mole fraction  $x_0$ ),

$$\frac{f_i e}{x_0} = \frac{P_{i+1}}{P_i} = f_s, \quad . \quad . \quad . \quad (32)$$

i.e.,  $f_i e$  and  $f_s$  are measured from the same zero of energy.

The system of equations appearing in their theory then takes the form

$$J = \frac{kT}{h} \left( x \omega_i \frac{P_{i+1}^{\dagger}}{P_i f_i e} \right) Z_i - \frac{kT}{h} \omega_{i+1} \frac{P_{i+1}^{\dagger}}{P_{i+1}} Z_{i+1} \quad . \quad . \quad (33)$$

From these, there results

$$J = Z_1 \left\{ \sum_1^{mlh} \left( \frac{kT}{h} \omega_1 \frac{P_{1+1}^{\dagger}}{P_1} \left[ \frac{x}{f_1 e} \right]^{mlh} \right)^{-1} \right\} \quad . \quad . \quad (34)$$

The partition function of the transition state can be written as

$$P_{1+1}^{\dagger} = P_{mlh} f^{\dagger},$$

where  $f^{\dagger}$  is that part of the partition function due to the adsorbing molecule.

We may consider the small crystal to be composed of

$$(m-2)(h-2)(l-2)$$

interior molecules, each of which has a partition function of  $f'''_{mlh}$ ; of

$$2(m-2)(l-2) + 2(l-2)(h-2) + 2(h-2)(m-2)$$

surface molecules, each with a partition function of  $f_{mlh(s)}$ ; of

$$4(m+1+h)-8$$

edge molecules, each with a partition function of  $f_{mlh(e)}$ ; and finally of eight corner molecules, each with a partition function  $f_{mlh(c)}$ . Putting

$$\frac{f_{mlh(s)}}{f'''_{mlh}} = f''_{mlh}, \quad . \quad . \quad . \quad (35)$$

$$\frac{f_{mlh(e)}}{f'''_{mlh} f_{mlh(s)}} = f'_{mlh}, \quad . \quad . \quad . \quad (36)$$

$$\frac{f_{mlh(c)}}{f'''_{mlh} f_{mlh(s)} f_{mlh(e)}} = f^{\circ}_{mlh}, \quad . \quad . \quad . \quad (37)$$

we have

$$P_{mlh} = (f'''_{mlh})^{mlh} (f''_{mlh})^{2(ml+lh+hm)} (f'_{mlh})^{4(m+l+h)} (f^{\circ}_{mlh})^8.$$

The condition for quasi-equilibrium between the supersaturated solution and the small crystal is given by

$$\frac{x}{f_{ie}} = \frac{P_{mlh}}{P_{mlh+1}} = \frac{1}{f'''(f'')^{4/n^{1/2}}(f')^{4/n^{1/2}}\delta} \quad (38)$$

where  $\delta$  takes into account the changes in the partition functions  $f_{mlh}$  when  $mlh$  is increased by unity and  $n = mlh$ . Inserting this value of  $\frac{x}{f_{ie}}$  into the equation for  $J$ , and taking  $m = l = h = 2\kappa$ , we obtain

$$J = Z_1 \frac{kT}{h} \omega_1 \frac{(f'')^{2n^{1/2}}(f')^{8n^{1/2}}(f^0)^{8f^{\dagger}\delta}}{P_1} \quad (39)$$

Also 
$$\frac{Z_1}{P_1} = \frac{N_0 x}{f_{ie}}.$$

All the terms except  $f^{\dagger}$  and  $\delta$  appear in different guise in the Becker-Döring theory, so it is these two terms we must examine. The free energy change for the deposition of the last molecule into the activated adsorbed state on the crystal  $mlh$  is given by

$$-\Delta F^{\dagger} = kT \log \frac{f^{\dagger} x}{f_{ie}} \quad (40)$$

The entropy portion of this change will be due mainly to the loss of the rotational degrees of freedom of the dissolved molecule as it becomes activatedly adsorbed. Since such a complicated molecule as cyclonite must fit precisely into the lattice, this entropy factor may reduce the probability of nucleation by as much<sup>10</sup> as  $10^{-10}$ . The factor  $\delta$  takes into account the fact that the molecular partition functions in the small crystal vary with the size of the crystal. For example, the oscillation frequency of a molecule in a small crystal will be greater than that in an infinite crystal. The entropy change due to this can be estimated from the decrease in entropy of a crystal under the influence of increased pressure. This is given by

$$-\Delta S = \int \frac{\partial V}{\partial T} dp \quad (41)$$

However, if likely values of the coefficient of expansion and the pressure change are substituted, the effect proves to be insignificant.

It is possible that an examination of the partition functions of small crystals might reveal interesting factors, since the distribution of the normal modes may be expected to depend upon the size to a very marked extent. Another possibility is that the nucleus may not at the critical stage be a small crystal, but may partake of some of the properties approximating to a liquid. A variation of this idea would be a suggestion that the adsorbed monolayer is non-localized.

We wish to thank Prof. W. E. Garner, F.R.S., under whose direction this work was carried out, for his interest and encouragement, and also Prof. E. G. Cox, Dr. A. Brewin and Dr. M. Hey for helpful discussion.

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<sup>10</sup> Eyring, ref. 9, p. 19.

# KINETICS OF CRYSTALLIZATION

## Part II

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In Part I, the continuous method of studying crystallization kinetics was discussed and some results were presented. Although the results obtained are extremely promising, the state of crystallization as a branch of knowledge is so insecure that we consider it necessary to develop a number of different modes of experimental attack. If, then, results are obtained by widely different techniques, any agreement among them can be considered significant. Criticisms can often be made against experiments the validity of which is difficult to assess. For example, one obvious objection to the continuous process is that the crystals, already present in the stirred vessel, "catalyze" in some way the formation of new nuclei, e.g., small submicroscopic chips may be formed by mutual attrition. Again, in the continuous process it is difficult to exclude foreign nuclei without considerable elaboration. Whilst, if the results are precise, it is possible to exclude such criticisms by a study of the functional dependence on the variables, nevertheless we have considered other methods of investigation, the results of which can be used to check those from the continuous process.

Another such technique can be referred to as the "batch process" to distinguish it from the continuous process. In this method a degree of supersaturation is brought about suddenly, giving rise to subsequent nucleation and growth. The initial supersaturation then falls to zero because of the precipitation of the new phase. In the experiments to be described, the initial supersaturation was brought about by the addition of water to a concentrated solution of cyclonite in acetone. The solubility of cyclonite is much less in the aqueous acetone. It is profitable to consider the mathematics of such a precipitation. It will be assumed that the rate of nucleation depends only upon the supersaturation  $S$  (where

$$S = \frac{c_i - c_{i0}}{c_{i0}},$$

$c_i$  is the concentration of the supersaturated solution and  $c_{i0}$  that of the saturated solution) and not upon the presence or absence of other crystals, nor upon such effects as stirring. In this case we can put:

$$\text{Rate of nucleation} = F(S), \quad (1)$$

where  $F(S)$  is an unspecified function of the supersaturation. In the same way we will assume that the linear rate of growth of nuclei and crystals is also a function of  $S$  alone and does not depend upon the size, rate of stirring, etc. Hence, if  $r$  is a linear dimension,

$$dr/d\theta = f(S), \quad (2)$$

where  $\theta$  is the time measured from the initiation of supersaturation. At such a time  $\theta$ , the distribution of size of the crystals in suspension, can be described by a function

$$n(r, \theta), \quad (3)$$

which gives the number of crystals present at time  $\theta$  that have radii greater than  $r$ . In this notation, the rate of nucleation is

$$\frac{\partial n(o, \theta)}{\partial \theta} = F(S) \quad . \quad . \quad . \quad (4)$$

Since only nuclei with  $r = 0$  are newly formed, the total differential of  $n(r, \theta)$  with respect to  $\theta$  will be zero, i.e.,

$$\frac{\partial n(r, \theta)}{\partial \theta} + \frac{\partial n(r, \theta)}{\partial r} \cdot \frac{\partial r}{\partial \theta} = 0 \quad . \quad . \quad . \quad (5)$$

or

$$\frac{\partial n(r, \theta)}{\partial \theta} = -f(S) \cdot \frac{\partial n(r, \theta)}{\partial r};$$

finally the supersaturation  $S(\theta)$  at time  $\theta$  is equal to the initial supersaturation  $S(o)$ , less the amount of solid which has crystallized at time  $\theta$ , i.e.,

$$S(\theta) = S(o) - \frac{\omega d}{M} \int_0^{\theta} \frac{\partial n(r, \theta)}{\partial r} \cdot r^3 dr, \quad . \quad . \quad . \quad (6)$$

where  $d$  is the density,  $M$  the molecular weight,  $r(\theta)$  the radius of the largest crystals, i.e., those which were born when  $\theta = 0$ , and  $\omega$  is a shape factor ( $= 4\pi/3$  for spherical crystals). We may also write

$$S(\theta) = S(o) - \frac{\omega d}{M} \int_0^{\theta} \frac{\partial n(o, t)}{\partial t} \left\{ \int_t^{\theta} f(S_r) \cdot d\tau \right\}^3 dt, \quad . \quad . \quad (7)$$

$$\text{i.e.,} \quad S(\theta) = S(o) - \frac{\omega d}{M} \int_0^{\theta} F(S_t) \cdot \left\{ \int_t^{\theta} f(S_r) d\tau \right\}^3 dt, \quad . \quad . \quad (7a)$$

where  $0 < t < \theta$  and  $t < \tau < \theta$ .

We now consider applications of these expressions to experiments. If the experiment consists of following the decrease of the supersaturation with time,  $S(\theta)$  is then known, but to derive the functions  $F(S)$  and  $f(S)$  from this relation would be a laborious task, unless the functions are of very simple form.<sup>1</sup> Even if the general forms of the functions are assumed to be those given by the Becker-Döring theory,<sup>2</sup> it would still be a lengthy task to match up the two sides of the equation since the surface free energies and edge free energies are not in general known. A more promising method would be to consider the relation in neighbourhood of  $S(o)$ . We can then put as a first approximation on the right-hand side of (7a),

$$S_t = S_r = S(o),$$

$$\text{and obtain} \quad S(\theta) - S(o) = \frac{\omega d}{M} \cdot F\{S(o)\} \cdot [f\{S(o)\}]^3 \cdot \theta^4 \quad . \quad . \quad (8)$$

If, therefore,  $S(\theta)$  is plotted against  $\theta^4$ , the tangent at  $S(o)$  will give a value of

$$F\{S(o)\} \cdot [f\{S(o)\}]^3$$

and the form of this product can be obtained from a series of experiments in which  $S(o)$  is varied.

Another type of experiment is that in which the increase in the Tyndall scattering of the crystallizing solution is observed. A beam of light is passed through the solution and the intensity of the scattered light measured throughout the course of the precipitation. In the initial stages of the

<sup>1</sup> Todes, *Acta Physicochim.*, 1940, **13**, 617.

<sup>2</sup> Becker and Döring, *Ann. Physik*, 1935, **24**, 719.

precipitation, the particles will be small enough to scatter light according to Rayleigh's formula,<sup>3</sup>

$$I = KI_0 (1 + \cos^2 \varphi) \frac{n.v^2}{\lambda^4}, \quad (9)$$

where  $I$  is the intensity of the scattered light of wavelength  $\lambda$  and primary intensity  $I_0$ , measured at an angle  $\varphi$ , the number of particles of volume  $v$  being  $n$  per unit volume. At time  $\theta$ ,

$$\sum n.v^2 = \frac{4\pi}{3} \int_0^\theta F(S_t) \cdot \left[ \int_t^\theta f(S_\tau) \cdot d\tau \right]^6 \cdot dt \quad (10)$$

assuming spherical particles. Hence

$$I(\theta) = \frac{K \cdot I_0 (1 + \cos^2 \varphi) \cdot 4\pi}{3\lambda^4} F\{S(\theta)\} [f\{S(\theta)\}]^6 \cdot \theta^7 \quad (11)$$

when  $\theta$  is small. From this relation it may be possible to derive values for

$$F\{S(\theta)\} [f\{S(\theta)\}]^6$$

for various initial supersaturations. Carried out in conjunction with the previous experiment in which

$$F\{S(\theta)\} [f\{S(\theta)\}]^3$$

is found both  $F\{S(\theta)\}$  and  $f\{S(\theta)\}$  should be derivable. Such a technique should give valuable information regarding the initial stages of formation and growth of nuclei. When the crystals grow larger and hence comparable with the wavelength of the light used, deviations from the Rayleigh expression will appear.<sup>4</sup> Observations on the polarization of the scattered light would furnish some information on the shape of the nuclei.

Another method of obtaining  $F(S)$  and  $f(S)$  has been studied by us, in which the final particle size distribution of the precipitated crystals has been used. For  $\theta = \infty$  eqn. (6) gives

$$S(0) - S(\infty) = \frac{\omega d}{M} \int_0^{r_{\max.}} \frac{\partial n(r, \infty)}{\partial r} \cdot r^3 dr, \quad (12)$$

where  $\frac{\partial n(r, \infty)}{\partial r}$  is the size distribution of the final precipitate\* and  $r_{\max.}$  is the size of the largest crystals present. These largest crystals are those born first in the experiment and have therefore been growing for the longest time in the most supersaturated solution. Hence in the final particle size distribution, we may immediately identify the largest crystals present as resulting from those nuclei born in the time interval 0 to  $d\theta$  when the supersaturation was  $S(0)$ . In the same way, all particles in the range  $r_\theta$  to  $r_\theta + dr_\theta$  of the final distribution were born in the time interval  $\theta$  to  $\theta + d\theta$  when the supersaturation was  $S_\theta$ . Now the number of such crystals in the final distribution is

$$\left\{ \frac{\partial n(r, \infty)}{\partial r} \right\}_{r=r_\theta} \cdot dr, \quad (13)$$

which is equal to the number of nuclei born in the interval  $\theta, \theta + d\theta$ . Hence

$$\left\{ \frac{\partial n(r, \infty)}{\partial r} \right\}_{r=r_\theta} dr = \frac{\partial n(0, \theta)}{\partial \theta} d\theta \quad (14)$$

<sup>3</sup> Rayleigh, *Phil. Mag.*, 1899, **47**, 375.

<sup>4</sup> La Mer, *J. Physic. Chem.*, 1948, **52**, 65.

\* Note that the symbol here differs from that used in Part I, there  $n(r)$  was used for  $\frac{\partial n(r)}{\partial r}$ .

$$\text{or} \quad \left\{ \frac{\partial n(0, \theta)}{\partial \theta} \right\}_{r=r_\theta} = F\{S(\theta)\} = \left\{ \frac{\partial n(r, \infty)}{\partial r} \right\}_{r=r_\theta} \cdot \left\{ \frac{dr}{d\theta} \right\}_{r=r_\theta} \quad (15)$$

$$= \left\{ \frac{\partial n(r, \infty)}{\partial r} \right\}_{r=r_\theta} \cdot f\{S(\theta)\} \quad (16)$$

$$\text{and} \quad \frac{F\{S(\theta)\}}{f\{S(\theta)\}} = \left\{ \frac{\partial n(r, \infty)}{\partial r} \right\}_{r=r_\theta} \quad (17)$$

The right-hand side of the equation is known from the determined particle size distribution, and so the ratio on the left is known.

The supersaturation  $S(\theta)$  at which these particles were born can be calculated. From (7a) and (15) we have

$$S(\theta) = S(0) - \frac{\omega d}{M} \int_0^\theta \left\{ \frac{\partial n(r, \infty)}{\partial r} \right\}_{r=r_i} \cdot f\{S(t)\} \left[ \int f\{S_\tau\} \cdot d\tau \right]^3 dt;$$

using (2) we have

$$S(\theta) = S(0) - \frac{\omega d}{M} \int_0^\theta \left\{ \frac{\partial n(r, \infty)}{\partial r} \right\}_{r=r_i} \cdot \left[ \int_i^\theta f\{S_\tau\} \cdot d\tau \right]^3 \cdot dr_i,$$

and if the rate of growth is independent of the size then

$$\int_i^\theta f\{S_\tau\} d\tau = \int_{r_{\max}}^r dr;$$

finally,

$$S(\theta) = S(0) - \frac{\omega d}{M} \int_{r_{\max}}^{r_\theta} \left\{ \frac{\partial n(r, \infty)}{\partial r} \right\}_{r_\theta < r < r_{\max}} \cdot \left[ \int_{r_{\max}}^r dr \right]^3 \cdot dr \quad (18)$$

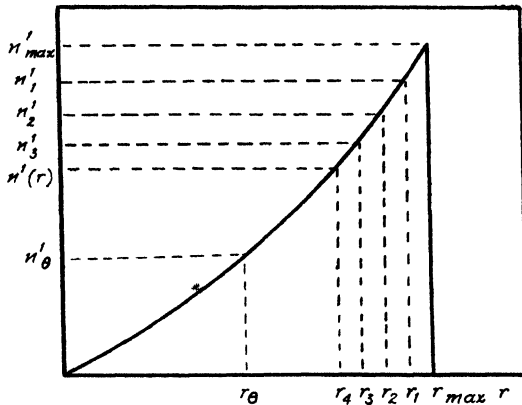


FIG. 1.

The integration of (18) can be carried out graphically quite readily from the final particle size distribution. The photoelectric sedimentometer gives a plot of  $\frac{\partial n(r, \infty)}{\partial r}$  against  $r$ . In Fig. 1 we have put  $\frac{\partial n(r, \infty)}{\partial r}$  as  $n'(r)$  in closer conformity with our notation in Part I. To illustrate the procedure in the graphical integration of eqn. (18) abscissæ  $r_{\max}$ ,  $r_1$ ,  $r_2$ ,  $r_3$ ,  $r_4$  . . . . ., and



the corresponding ordinates,  $n'_{\max.}$ ,  $n'_1$ ,  $n'_2$ ,  $n'_3$ ,  $n'_4 \dots$ , are marked off. The supersaturations at which these groups were born are then obtained as

$$\begin{aligned} S(0) - S_1 &= \frac{\omega d}{M} \cdot n'_1 \cdot (r_{\max.} - r_1)^3, \\ S(0) - S_2 &= \frac{\omega d}{M} \left\{ n'_1 (r_{\max.} - r_2)^3 + n'_2 (r_{\max.} - r_1)^3 \right\}, \\ S(0) - S(\theta) &= \frac{\omega d}{M} \left\{ n'_1 (r_{\max.} - r_\theta)^3 + \dots + n'_\theta (r_{\max.} - r_1)^3 \right\}. \end{aligned}$$

In this manner we can obtain the ratio  $\frac{F\{S(\theta)\}}{f\{S(\theta)\}}$  ( $= n'_\theta$  in Fig. 1), as a function of  $S_\theta$  from a single precipitation. In order to separate  $F\{S(\theta)\}$  from  $f\{S(\theta)\}$ , the following technique appears to be available. From eqn. (7a),

$$-\left(\frac{dS}{d\theta}\right)_\theta = \frac{3\omega d}{M} \cdot f\{S(\theta)\} \cdot \int_0^\theta F(S_t) \cdot \left[ \int_t^\theta f(S_\tau) \cdot d\tau \right]^2 dt,$$

but 
$$A(\theta) = \varphi \int_0^\theta F(S_t) \cdot \left[ \int_t^\theta f(S_\tau) d\tau \right]^2 dt,$$

where  $A(\theta)$  is the total surface area of the precipitate present at time  $\theta$  and  $\varphi$  is a shape factor ( $= 4\pi$  for spheres). Hence

$$-\left(\frac{dS}{d\theta}\right)_\theta = A(\theta) \cdot f\{S(\theta)\}.$$

If, therefore, during the experiment the changes in the supersaturation and the total surface area of the precipitate can be recorded, then  $f\{S(\theta)\}$  can be evaluated. A convenient method of determining  $A(\theta)$  is by means of a photoelectric turbidimeter (similar in operation to our photoelectric sedimentometer<sup>5</sup>).

### Experimental

A solution of recrystallized cyclonite in acetone-water was prepared and freed from foreign nuclei by developing these slowly to filterable size. More solvent was then added to the filtered solutions and they were then kept at about 35° C for some hours before use. The solution was transferred to the jacketed vessel A (Fig. 2); B and C form a second smaller vessel, and C is a flat plate attached to a shaft; it carries paddles on its outer edge, which stir the contents of vessel A when the shaft rotates. B is a composite tube consisting of a narrow plate fitting over the shaft of C and a wider portion at the bottom. The end of this wide portion was ground and polished to fit the surface of plate C, so that when pressed against the plate the two form a liquid-tight vessel. It was found necessary to grease the joint slightly in order to render the small vessel liquid-tight. The tube B was attached to a collar sliding on the shaft of C and driven round with the shaft by a sliding key. This small vessel contained sufficient water to dilute the solution in A to a final concentration of 50 % by weight. After the two liquids had reached the temperature of the circulating water (about 24.7° C), a trigger was released and the tube B was snapped away from the plate C by means of a spring. This occurred with the stirrer shaft in motion and the diluting water was projected and stirred rapidly into the outer vessel. A small heat rise (about 0.3° C) occurred on mixing and so water from a second thermostat at 25° C was switched in at the moment of mixing.

In this apparatus there was no means of following the decrease of supersaturation, and visual observation was relied upon to estimate when the precipitation had approached completion. The crystals were then filtered by means of

<sup>5</sup> Bransom and Dunning, *J. Soc. Chem. Ind.* 1949, **68**, 80.

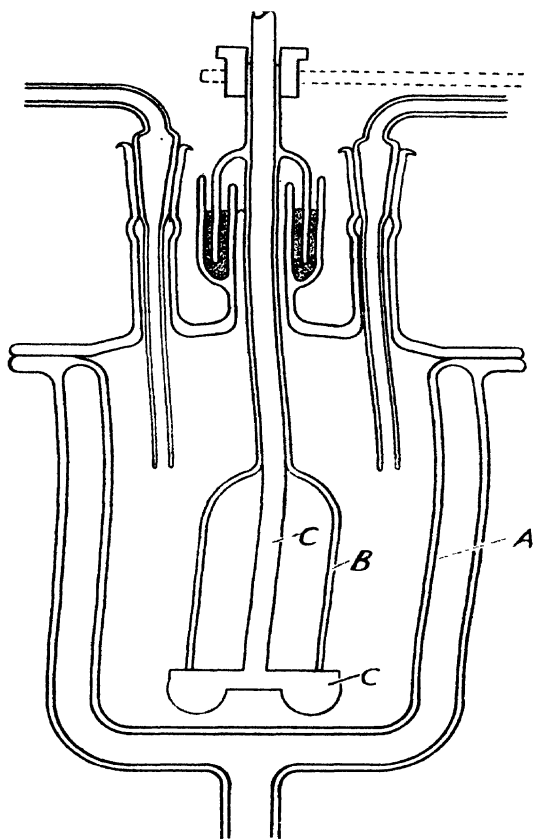


FIG. 2.

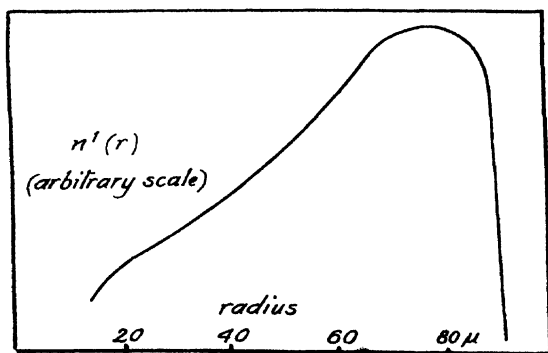


FIG. 3.

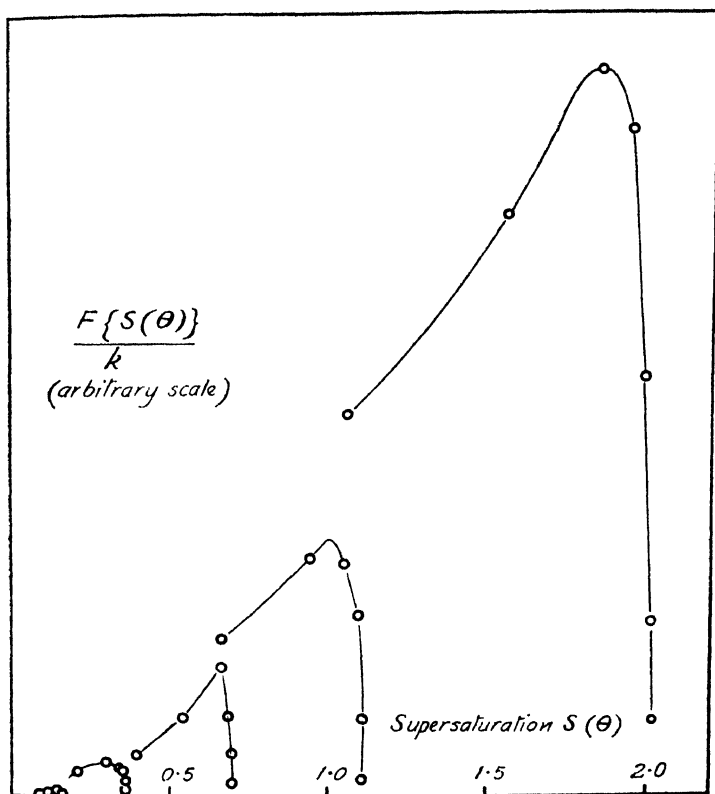


FIG. 4.

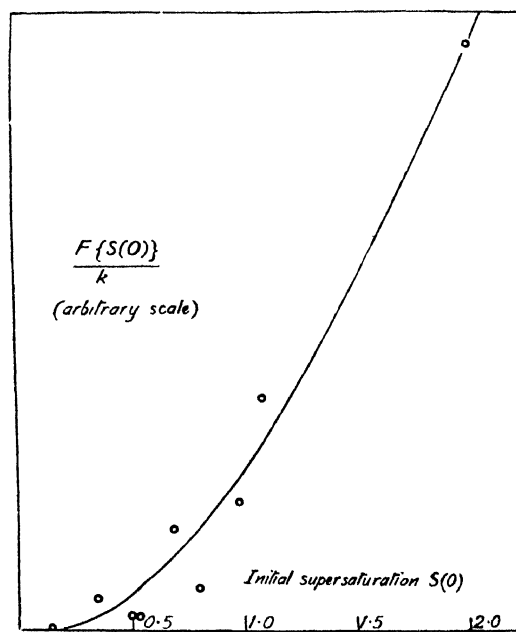


FIG. 5.

a jacketed filter and the concentration of the solute in the mother liquor was determined and checked against the predetermined solubility. The crystals were weighed and a sample analyzed for particle size distribution.

Fig. 3 illustrates the type of size distribution for colonies produced from solutions whose initial supersaturations were less than about 2.5. The number of particles  $n'(r)$  with sizes between  $r$  and  $r + dr$  rises to maximum with increasing  $r$  and then drops very steeply to zero. Theoretical considerations suggest that there should be a sharp cusp at the cut-off at  $r_{\max.}$ , the rounding of the curve at this point which is found experimentally is no doubt due to limitations in the experimental technique. The cut-off in the region of small radii is probably due to the loss of the smaller crystals through the filter.

These size distributions were treated as described above in order to obtain  $\frac{F\{S(\theta)\}}{f\{S(\theta)\}}$  as a function of  $S_\theta$ , and it was further assumed that

$$f\{S(\theta)\} = kS(\theta),$$

where  $k$  is a constant. In this way, by multiplying by  $S(\theta)$ , values of  $\frac{F\{S(\theta)\}}{k}$  were obtained. Fig. 4 gives the plots of these values as derived from a series of experiments in which the initial supersaturations were varied.

It is seen that the curves superimpose on each other. This implies that the rate of nucleation depends only upon the supersaturation and not upon the presence of crystals. Hence there is no evidence for auto- or secondary-nucleation,\* nor does the stirring cause attrition with the formation of small centres for crystallization. Furthermore, it is seen that the points for initial nucleations also lie on the curve. Since the initial supersaturations are known from the quantities of the solvents and solutions which were used, the integrations were dispensed with and the particle size distributions merely used to obtain the number of particles with the maximum radius. In this way the rates of initial nucleation in the initial supersaturations were determined for a number of solutions. The results are shown in Fig. 5, where  $F\{S(\theta)\}/k$  is plotted against  $S(\theta)$ . Since  $k$ , or better  $f\{S(\theta)\}$ , is not known, the ordinates are relative in magnitude. However, it is seen that  $F(S)/k$  is a steeply rising function of the supersaturation.

It has not been considered profitable to examine these relationships in greater detail, e.g., in relation to the Becker-Döring theory, since the results are preliminary and serve mainly to illustrate an experimental technique.

We wish to express our thanks to Prof. W. E. Garner, Prof. E. G. Cox, Dr. M. Hey and Dr. B. Touschek for the interest they have shown in the work. The paper is published by permission of the Chief Scientist, Ministry of Supply.

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\* Altberg and Lavrow, *Acta Physicochim.*, 1940, **13**, 725.

## THE PRECIPITATION OF SILVER CHLORIDE FROM AQUEOUS SOLUTIONS

### Part I

BY C. W. DAVIES AND A. L. JONES

*Received 26th January, 1949*

When a substance separates spontaneously from its supersaturated solution the kinetics of the process can be expected to be most complex, at any rate in the early stages; for the growth of existing nuclei and the formation of new ones may be proceeding concurrently, and the number, size and size distribution of the crystal nuclei may be changing rapidly with

time. The experimental study is further complicated by the difficulty of exactly reproducing the conditions under which spontaneous nuclei formation is induced, either by gradual temperature variation or by the mixing of solutions; and a foreign substance present in traces, solid or dissolved, may be expected to exert an influence out of proportion to its concentration.

In view of all this it is not surprising that the published measurements of crystallization rates have been difficult to interpret, and that such theories as have been advanced still lack convincing experimental support. In our work we have tried to simplify the problem by first studying the behaviour of silver chloride under such conditions of concentration that fresh nuclei are not being formed. The measurements reported here help to define these conditions.

### Experimental

Precipitation was followed by measuring the conductivity of the solution. The amount of AgCl precipitated at any moment was calculated from the equation:

$$-\Delta\kappa = -\frac{\Lambda \cdot \Delta C}{1000} = -\frac{\Lambda \cdot \Delta n}{v}$$

where  $n$  is the number of g.-moles precipitated,  $\Delta\kappa$  the corresponding change in the specific conductivity of the solution,  $v$  the volume of solution in ml. and  $\Lambda$  the sum of the ionic conductances of the silver and chloride ions. The latter quantity could be regarded as a constant, within experimental error, in the solutions investigated which were all within the range of ionic strength  $1 - 3 \times 10^{-3}$ ; it was given the value<sup>1</sup>:  $\Lambda = 138.26 - 91.7 (3 \times 10^{-3})^{1/2} = 137.76$ , where 91.7 is the Onsager slope. The concentration of KCl or AgNO<sub>3</sub>, when required, could be calculated in a similar way from the conductivities, or vice versa, using the mobility values<sup>1</sup>: 73.52 for K<sup>+</sup>, and 71.44 for NO<sub>3</sub><sup>-</sup>.

The measuring bridge was of the type previously described,<sup>2</sup> and, with the low conductivities studied in this work, enabled changes of the order of 0.01 % to be observed. The solutions were contained in silica or borosilicate cells of the Hartley and Barrett type with greyed platinum electrodes. One was fitted with an efficient Pyrex glass stirrer which could rotate below the electrodes; all experiments involving seed crystals were carried out in this cell, the normal stirring rate being 250–300 rev./min. The cells were immersed in an oil thermostat controlled at  $25^\circ \text{C} \pm 0.01^\circ$ , and the room was thermostated  $25^\circ \pm 1^\circ \text{C}$ . During runs the cells were either sealed, or a gentle stream of pure air or nitrogen was passed over the solutions; under these conditions blank experiments showed that variations in the CO<sub>2</sub>-content of the water—probably the largest single source of error in the measurements—could be controlled, and constant conductivities maintained over many hours. The cells were calibrated by measuring the conductivities of very dilute KCl solutions at  $25^\circ \text{C}$ , and applying the interpolation formula<sup>2</sup>:

$$\Lambda = 149.92 - 93.85 C^{1/2} + 50C.$$

The 'cell constants' so calculated varied from the mean value by not more than 0.04 %.

The water used for the experiments was obtained from a modified Bourdillon still; its specific conductivity varied between 0.2 and  $0.6 \times 10^{-6} \text{ ohm}^{-1}$ . KCl and AgNO<sub>3</sub> were both A.R. reagents. After every experiment the cell used was washed with ammonia to remove all traces of solid silver chloride.

Seed crystals of AgCl were prepared by crystallization from boiling, saturated solutions. Freshly precipitated AgCl was washed six times by decantation, portions of the precipitate were boiled in 2-l. volumes of distilled water and, after immediate filtration, the solution was allowed to cool very slowly in the dark. The crystals thus formed were washed with conductivity water, and suspensions made up and aged at  $25^\circ$  in darkness for at least a fortnight. The

<sup>1</sup> MacInnes, Shedlovsky and Longworth, *J. Amer. Chem. Soc.*, 1932, **54**, 2758.

<sup>2</sup> Davies, *J. Chem. Soc.*, 1937, 432.

seed concentrations were determined gravimetrically with a 1 % accuracy and were arranged to be of the order of 1 mg. AgCl per ml. suspension. Microscopic examination showed that the seed crystals were rectangular plates or cubes having an average size of 5-10  $\mu$ , and occurred singly or in very small clusters.

### Results

**The Solubility Product at 25° C.**—In these determinations the equilibrium was approached from both sides. The precipitation experiments were arranged so that precipitation should occur only on the aged seed crystals. A stable supersaturated solution (see later) of AgCl was prepared in the cell, and CO<sub>2</sub>-free air passed through until the conductivity became constant. A known volume of a homogeneous seed suspension was then added, and the resulting decrease in conductivity followed to the equilibrium value, from which the solubility product of AgCl was calculated. The results are in Table I.

TABLE I

No. of Expt.	Initial concn. $\times 10^5$		Ml. seed added	Duration of expt. (hr.)	Final concn. $\times 10^5$		$S \times 10^{10}$
	[Ag <sup>+</sup> ]	[Cl <sup>-</sup> ]			[Ag <sup>+</sup> ]	[Cl <sup>-</sup> ]	
45	1.640	1.610	2.0	18	1.372	1.342	1.84
76	1.673	1.690	5.0	6	1.354	1.371	1.85
78	1.613	1.623	5.0	5	1.339	1.349	1.81
86	2.161	1.097	5.0	12	1.978	0.914	1.81
87	3.023	0.775	5.0	11	2.875	0.627	1.80

In a second series of experiments, larger quantities of seed were added to water of known conductivity in the cell, and the process of solution followed to apparent equilibrium. The solubility of AgCl was calculated from the final conductivity. A correction for traces of impurity in the seed suspension was obtained by adding further amounts of suspension after equilibrium had been attained, and noting any resulting change in the conductivity. The correction never exceeded 0.7 %. The results are given in Table II.

TABLE II

No. of Expt.	$\chi_{H_2O} \times 10^6$	Ml. seed added	Duration of expt. (hr.)	$\chi_{AgCl} \times 10^6$ (corr.)	$S \times 10^{10}$
53	0.365	10	48	1.873	1.84
54	0.456	14	48	1.848	1.80
80	0.304	5	40	1.844	1.79
81	0.431	7	46	1.849	1.80

The suspension used in Expt. 53 and 54 was four times, and that of Expt. 80 and 81 ten times, more concentrated than that used in the experiments of Table I. The two methods are in good agreement, and give  $1.82 \times 10^{-10} \pm 0.02$  for the concentration solubility product, or  $1.35 \times 10^{-5} \pm 0.01$  g.-mole/l. for the solubility of silver chloride at 25° C. This value is confirmed by a third method to be described in Part II. Introducing activity coefficients calculated from the Debye-Hückel limiting equation the true (activity) solubility product becomes  $1.81 \times 10^{-10}$ . Landolt-Börnstein<sup>3</sup> quotes nine previous determinations of the solubility ranging from 1.20 to  $1.47 \times 10^{-5}$  g.-mole/l.; the average of these values is also  $1.35 \times 10^{-5}$ .

<sup>3</sup> Landolt-Börnstein, *Physik.-Chem. Tabellen* (Springer, Berlin 5te. Aufl., 1923), 634 ; Erg. II, 343 ; Erg. III, 483.

**Precipitations in the absence of seed.**—Precipitation may be initiated by bringing an  $\text{AgNO}_3$  solution of the approximate concentration  $1.35 \times 10^{-5}$  to temperature equilibrium in the cell, and then adding from a weight burette a sufficient quantity of a more concentrated  $\text{KCl}$  solution to exceed the solubility product. Three such experiments are illustrated in Fig. 1. Curve 1 refers to a run in which 2 ml. 0.004 N  $\text{KCl}$  solution were added, curve 2 to one in which 10 ml. 0.0008 N solution were used and curve 3 to one in which 20 ml. 0.0004 N solution were employed; in this last case the  $\text{KCl}$  was added in portions over a considerable period of time, the final addition of 1 ml. producing precipitation. The three curves are strikingly different, and it is clear that the course of the precipitation is governed mainly by the local concentrations of the ions at the moment of mixing. The number of nuclei available for the subsequent separation of  $\text{AgCl}$  is greatest for the experiment of curve 1 and least for curve 3. It may be added that runs carried out in this way are not reproducible, and the final conductivities reached after many hours always correspond to solubility products greater than  $1.82 \times 10^{-10}$ , indicating that the resulting crystals are small enough to show an enhanced solubility. Curve 1 gave a final concentration product of  $2.02 \times 10^{-10}$  and curve 2 a value of  $1.93 \times 10^{-10}$ , in agreement with the view that more nuclei were available in the faster run which resulted from the higher local concentration on mixing. The run corresponding to curve 3 was not complete after 40 hr.

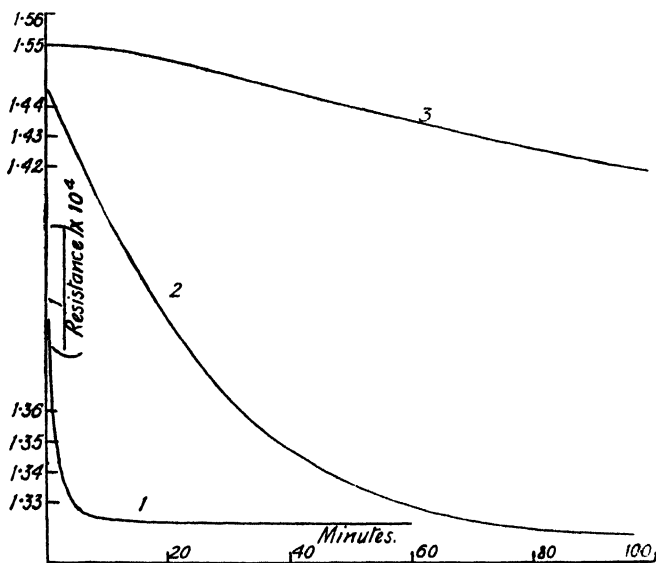


FIG. 1.—Precipitations from unseeded solutions.

To avoid these local concentration effects it is necessary to mix solutions of approximately equal concentrations, and a number of experiments were made in this way. The cell was half-filled with a solution of one of the reagents, and when temperature equilibrium had been established an equal volume of the other reagent, preheated to  $25^\circ$ , was introduced and the cell gently shaken. A few runs of short duration were inconclusive, but Table III contains the results of all experiments by this technique which were followed for at least three hours.

In Expt. 28-1 the total fall in conductivity over 12 hr. corresponded to a change of no more than 2 mm. in the bridge null-point, and was within the possible experimental error. With this exception all the solutions with a concentration product of  $3.14 \times 10^{-10}$  or less showed no precipitation after periods extending up to 18 hr., and this was true whether the solutions were mechanically stirred, occasionally shaken or agitated by the passage of a rapid stream of nitrogen. The experiments suggest therefore that such solutions, in which the solubility

TABLE III

No. of Expt.	Initial concn. $\times 10^6$		$[Ag^+][Cl^-] \times 10^{10}$	Duration (hr.)	$-\Delta\kappa \times 10^6/\text{hr.}$
	$[Ag^+]$	$[Cl^-]$			
36	1.88	1.48	2.80	15	0
24-3	1.71	1.71	2.92	7	0
28-2	1.75 <sub>s</sub>	1.75 <sub>s</sub>	3.08	9	0
24-B	1.76	1.76	3.10	14	0
28-1	1.76	1.76	3.10	12	0.003
24-C	1.78	1.76	3.14	18	0
28-3	1.78	1.78	3.16	8	0.008
24-D	1.79	1.77	3.17	7 $\frac{1}{2}$	0.009
24-6	1.80	1.80	3.24	19	0.013
24-4	1.88	1.88	3.53	2	0.081
31	1.86	1.89	3.53	3	0.080
35	1.96	1.94	3.80	40	0.11
24-2	2.09	2.09	4.37	3	0.26

product is exceeded by more than 50 %, will remain almost indefinitely without any crystal formation. The critical concentration product above which nuclei development occurs spontaneously is clearly very near  $3.14 \times 10^{-10}$ . To help in fixing it more closely we have used the data for the very slow precipitations which occur when the concentration product just exceeds this value. The last column of Table III gives the average rate of fall in conductivity over the first hour or two after mixing. These values are not very accurate, as the smallest of them is not much greater than the possible experimental error, whilst for the fastest runs the values begin to depend on the time interval chosen. Nevertheless, when plotted, as shown in the left-hand curve of Fig. 2, they give a reasonably good straight line which fixes the critical concentration product at  $3.14 \times 10^{-10}$ .

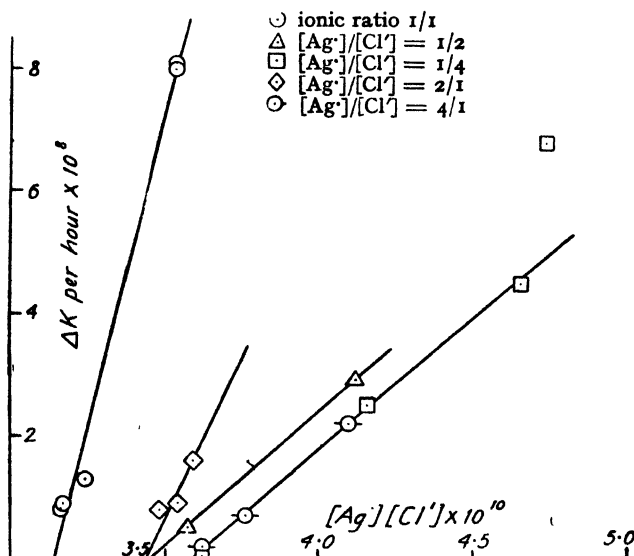


FIG. 2.—Rates of slow precipitations against ionic concentration product.

**The effect of ionic ratio on the critical supersaturation.**—Further series of experiments similar to those described in the last section have been carried out with initial  $[Ag^+]/[Cl^-]$  ratios of 0.25, 0.5, 2 and 4. These show quite definitely that a critical supersaturation limit exists in each case, but that its value depends



## PRECIPITATION OF SILVER CHLORIDE

on the ionic ratio. The results for a 2/1 ratio are the same (within experimental error) whichever ion is in excess, and the same is true for a 4/1 ratio. Mixtures which failed to show any precipitation are listed briefly in Table IV.

TABLE IV

Approx. ratio [Ag <sup>+</sup> ]/[Cl <sup>-</sup> ]	Concn. product × 10 <sup>10</sup>	Duration (hr.)	Approx. ratio [Ag <sup>+</sup> ]/[Cl <sup>-</sup> ]	Concn. product × 10 <sup>10</sup>	Duration (hr.)
1/2	3.15	3	2/1	3.38	9
	3.29	6		3.41	5
	3.37	5		3.52	14
	3.42	14		3.56	5

It will be seen that the critical supersaturation is increased by a disparity in the concentrations of silver and chloride ions. To fix the critical values for each concentration ratio more closely, further slow precipitations were carried out, and the results of these are shown in Fig. 2. They lead to the following values—

Ionic ratio	.. ..	1/1	1/2	1/4
Critical concn. product × 10 <sup>10</sup> ..	3.14	3.44	3.59	

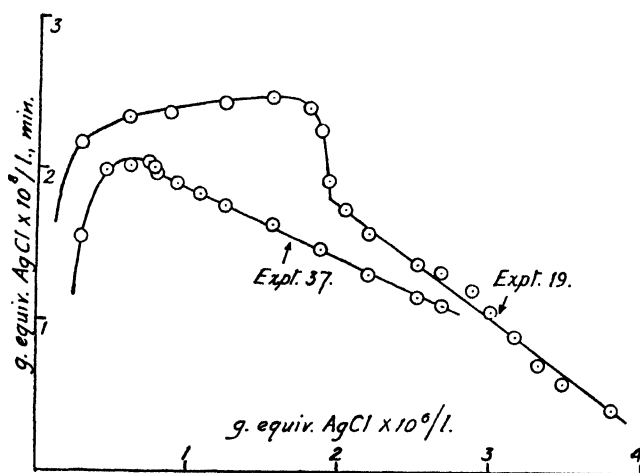


FIG. 3.—Rate of precipitation in g.-equiv. × 10<sup>8</sup>/l. min. plotted against amount precipitated in g.-equiv. × 10<sup>6</sup>/l.

Finally, some further evidence concerning the effect of ionic ratio has been obtained in an entirely different way. A number of moderately slow precipitation runs, of the type illustrated in curve 3 of Fig. 1, have been carried out and from the conductivity-time plots curves have been constructed showing the rate of precipitation plotted against the amount of silver chloride precipitated. Two of these are illustrated in Fig. 3; they show that the precipitation accelerates to a maximum, and that shortly after this the rate curve changes, at a fairly well-defined point, to a steady linear (or almost linear) decrease. We were inclined to interpret these turning-points as representing the stage in the precipitation at which fresh nuclei cease to be formed. If this is so, the concentration product at the turning-point may be identified with the critical supersaturation for the ionic ratio holding at the turning-point. Critical values based on this

hypothesis are compared in Fig. 4 with the directly determined values, and the agreement is excellent. It should be added that in the runs to which this method has been applied the turning-point is not reached until more than an hour after mixing the reagents. More rapid runs have shown fairly abrupt turning-points in their rate curves, but these have not corresponded with the directly determined critical supersaturations.

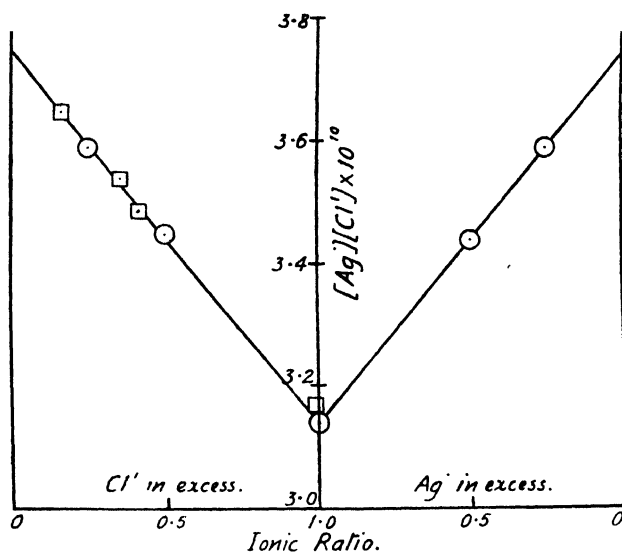


FIG. 4.—Critical supersaturations: ○ from direct determinations; □ from turning points.

### Discussion

The behaviour at 25° C of unseeded supersaturated solutions of silver chloride may be summarized as follows.

1. Spontaneous precipitation will not occur unless the product of the ionic concentrations is almost double the normal solubility product.
2. The value of this critical supersaturation is markedly dependent on the ionic ratio, but not on which ion is in excess. The data shown in Fig. 4 may be represented by the equation:

$$S_c \times 10^{10} = 3.74 - 0.60 n,$$

where  $S_c$  is the critical concentration product and  $n$  is the ionic ratio expressed as a fraction less than one.

3. If the critical concentration product is just exceeded precipitation always occurs, although it may be so slow as to be perceptible only after several hours. At slightly higher concentrations a characteristic rate curve is given from which, again, the critical supersaturation can be calculated. If the concentration is further increased the kinetics of precipitation become more complex, and when the initial concentrations are about ten times the normal solubility, the precipitation is almost instantaneous.

As is well known, Ostwald<sup>4</sup> believed that if the concentration of a solution be gradually increased, the region of stable unsaturated solution is followed, after the normal solubility curve, by a metastable region in which crystallization will not occur without suitable inoculation; and that this again is succeeded by a region of labile solutions which crystallize spontaneously.

<sup>4</sup> Ostwald, *Z. physik. Chem.*, 1897, **22**, 289.

This view was supported by the work of Miers,<sup>5</sup> Hartley,<sup>6</sup> Mouat Jones<sup>7</sup> and others, who plotted for many salts the course of the 'supersolubility curve' which separates the metastable from the labile region. It was criticized by de Coppet,<sup>8</sup> whose results were less regular, and who thought that sporadic crystallization was liable to occur, perhaps after long periods of time, in any supersaturated solution; and by Young,<sup>9</sup> who states that crystallization from the metastable region can always be induced by violent mechanical shock. In view of de Coppet and Young's criticisms, later writers<sup>10</sup> have tended to regard as unreal any rigid distinction between "labile" solutions (in which they consider that crystallization is rapid and easy) and "metastable" solutions (in which it is slow or more difficult), and this view has been quoted in a recent review.<sup>11</sup> It will be evident that our results support the earlier belief that metastable solutions can exist up to a definite limit, and this limit can be fixed with considerable accuracy and varies in a regular way with the composition (ionic ratio) of the solution: this applies to solutions under ordinary conditions; the abnormal conditions studied by Young introduce fresh considerations. Our results also differ from those of de Coppet in that silver chloride invariably precipitates even in unstirred solutions as soon as the supersolubility is exceeded.

The Gibbs-Thomson relation may be applied to the solubility of silver chloride particles in the form:

$$\ln \frac{[\text{Ag}]_1 [\text{Cl}]_1}{[\text{Ag}]_2 [\text{Cl}]_2} = \frac{\gamma V a}{RT} \left( \frac{1}{l_1} - \frac{1}{l_2} \right)$$

where  $\gamma$  is the interfacial tension,  $V$  the molecular volume of the solid salt,  $[\text{Ag}]_1 [\text{Cl}]_1$  the concentration product of a solution which is in equilibrium with crystals of (assumed uniform) average linear dimension  $l_1$ , and  $a$  is a numerical factor depending on the shape of the particles; when  $l_2$  becomes large,  $[\text{Ag}]_2 [\text{Cl}]_2$  becomes the normal solubility product. This equation cannot be used without a knowledge of the interfacial tension, and moreover it involves the assumption that the interfacial tension is independent of particle size; nevertheless it is qualitatively valid. It was used by Hartley and Thomas<sup>6</sup> to account for the metastable region. They assumed that crystal nuclei might not attain a size at which they could act as centres of further growth until the supersolubility curve was reached. This idea was extended by later workers,<sup>7</sup> so as to accommodate de Coppet's views, by supposing that chance encounters in the metastable range may occasionally give rise to a particle large enough to initiate crystallization.

To serve as a useful basis for discussion these conceptions must be stated with greater precision. If the concentration of a seed-free solution were uniformly increased through the normal solubility value, the rate of growth of any nuclei, however arising, would be increasingly favoured as compared with the rate of solution, until nuclei of a size satisfying the Gibbs-Thomson equation would be eventually produced. The corresponding concentration product would represent the critical supersaturation. Up to this point the nuclei would be unstable, the rate of loss by solution far exceeding, at first, the rate of molecular deposition, and we therefore think that the

<sup>5</sup> Miers, *Phil. Trans.*, 1904, **202**, 459. Miers and Isaac, *Proc. Roy. Soc. A*, 1907, **79**, 322; 1910, **82**, 184; *J. Chem. Soc.*, 1906, **86**, 413; 1908, **93**, 927.

<sup>6</sup> Hartley and Thomas, *J. Chem. Soc.*, 1906, **89**, 1013; Hartley, Jones and Hutchinson, *ibid.*, 1908, **93**, 825.

<sup>7</sup> Jones, *J. Chem. Soc.*, 1908, **93**, 1739; 1909, **95**, 1672.

<sup>8</sup> de Coppet, *Ann. Chim. Phys.*, 1907, **10**, 457.

<sup>9</sup> Young, *J. Amer. Chem. Soc.*, 1911, **33**, 148, 1375; 1913, **35**, 1067.

<sup>10</sup> Ting and McCabe, *Ind. Eng. Chem.*, 1934, **26**, 1201.

<sup>11</sup> Wells, *Ann. Reports*, 1946, **43**, 85.

main mechanism of growth is by the successive coalescence of smaller particles; it is only above the supersaturation point that this mechanism may give way to growth by molecular accretion. If this is correct, the number of nuclei attaining a given size will vary very rapidly with changes of concentration, and the critical supersaturation might be identified with a narrow range of concentration in which stable nuclei arise in significant numbers. A consequence of this view would be that stable nuclei can exist in small numbers even in the metastable region; but when we remember that a reduction of concentration will not only result in a very rapid drop in the number of nuclei of given size, but will also lead to a rapid increase in the minimum size of a stable nucleus, it is clear that the chance of detecting crystallization at a point well within the metastable region is vanishingly small.

This viewpoint is reconcilable with the results of Hartley, Jones *et al.* Our results have shown that if precipitation does not actually cease at the critical supersaturation, it must at least become so slow that no change would be detected over a period of days. And although the Oxford workers made many hundreds of experiments without once observing crystallization below their supersolubility curve, their method of cooling would not have enabled them to detect a very slow process.

It is possible that the theory also explains our own results: for we have no theoretical basis for the linear extrapolations of Fig. 2; and although we have shown that for all practical purposes the critical supersaturation is sharply defined, the experimental distinction can only be between solutions that do or do not show a perceptible change in a reasonable time.

#### ADDENDUM (13th April, 1949):

Further experiments in seeded solutions have now shown that the rate of crystal growth in slightly supersaturated solutions follows the equation:  $v = k\Delta^2$ , where  $v$  is the velocity of crystallization,  $k$  a constant, and  $\Delta$  is the quantity of silver and chloride ions to be deposited before equilibrium is attained. A result of this behaviour is that, for equal ionic concentration products, the rate of crystal growth will be the smaller the greater is the disparity between the individual ionic concentrations; the effect is illustrated by the following results for the initial rate of deposition from solutions in which the ionic concentration products are roughly equal:

$[\text{Ag}^+]/[\text{Cl}^-]$ .. ..	1	2	$\frac{1}{2}$	4	$\frac{1}{4}$
$[\text{Ag}^+][\text{Cl}^-] \times 10^{10}$ ..	2.605	2.585	2.560	2.588	2.560
$10^8 \times v$ .. ..	1.84	1.54	1.48	1.15	1.06
$10^{-3} \times v/\Delta^2$ ..	2.52	2.54	2.58	2.71	2.68

This provides an explanation of the ionic ratio effect illustrated in Fig. 4 (and it is no longer necessary to assume that coalescence plays a major part in nucleus formation); a nucleus of stable size grows more slowly if the ionic ratio is not unity, and the probability of a nucleus growing to stable size within a given limit of time should be reduced in a similar way.

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# NUCLEATION AND GROWTH IN SUCROSE SOLUTIONS

BY ANDREW VAN HOOK AND ARTHUR J. BRUNO

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Schweizer<sup>1</sup> has pointed out the difficulty of preparing, by ordinary procedures, supersaturated solutions of highly soluble materials which do not exhibit a tendency to nucleate spontaneously. He was able to prepare stable, supersaturated solutions of sucrose and other substances, which were 1.8-fold supersaturated on a sugar to water basis, and which did not crystallize over periods of several months. On the other hand, Waterman and Gentil<sup>2</sup> found that all oversaturated solutions of sucrose crystallized, given sufficient time. Both behaviours find support in the voluminous literature on the nucleation of solutions and melts, e.g., Cassel and Landt,<sup>3</sup> Kucharenko,<sup>4</sup> Meyer and Pfaff,<sup>5</sup> Dorsey,<sup>6</sup> Volmer,<sup>7</sup> Stranski,<sup>8</sup> etc., on the basis of the heterogeneous or thermodynamic theories; Richards,<sup>9</sup> Tammann,<sup>10</sup> Van Ginnekin and Smit,<sup>11</sup> Fouquet,<sup>12</sup> etc., on the basis of the homogeneous theory.

It is the purpose of this paper to review this situation for sucrose solutions, from the theoretical and practical viewpoints. Such matter has been considered previously by Cassel and Landt,<sup>3</sup> Naveau<sup>13</sup> and Capelle.<sup>14</sup>

**Preparation of Stable Supersaturated Sucrose Solutions.**—Both Schweizer's and Waterman and Gentil's experiences were confirmed, using their respective techniques. However, in the latter procedure, which involves dissolution in sealed tubes, it was observed to be possible to prepare slightly oversaturated syrups which have not crystallized over several months, if fine, alcohol-precipitated material was used and/or complete solution was assured by prolonged rotation at temperatures at least 20° above the saturation point. If these precautions are not observed, or if the supersaturation is too high (> 1.6), crystallization inevitably occurs.

It was likewise found possible to duplicate Schweizer's experience with solutions prepared by means of quick, active boiling, followed by curing (after sealing, or covering with a thick layer of Nujol oil) for at least 20 min. and 20° above the saturation point of the final solution. Presumably the potential nuclei, otherwise preserved, are deactivated by this treatment. One is limited to prepare, at most, approximately 80 % solutions by either technique; since beyond this concentration either degradation is unavoidable

<sup>1</sup> Schweizer, *Rec. trav. chim.*, 1933, **52**, 678; *Int. Sugar J.*, 1933, **35**, 385.

<sup>2</sup> Waterman and Gentil, *Chem. Weekblad*, 1926, **23**, 345.

<sup>3</sup> Cassel and Landt, *Z. dtsch. Zucker-Ind.*, 1927, **77**, 483.

<sup>4</sup> Kucharenko, *Planter Sugar Mfg.*, 1928, **75**.

<sup>5</sup> Meyer and Pfaff, *Z. anorg. Chem.*, **217**, 257; **222**, 382; **224**, 305.

<sup>6</sup> Dorsey, *Trans. Amer. Phil. Soc.*, 1948, **38**, 248.

<sup>7</sup> Volmer, *Kinetik der Phasenbildung* (Steinkopff, Dresden, 1939).

<sup>8</sup> Stranski, *Physik. Z.*, **36**, 393; *Ann. Physik*, **23**, 330.

<sup>9</sup> Richards, *J. Amer. Chem. Soc.*, 1936, **58**, 2243.

<sup>10</sup> Tammann, *Kristallisieren und Schmelzen* (Barth, Leipzig, 1903); *States of Aggregation* (Van Nostrand, N.Y., 1925).

<sup>11</sup> Van Ginnekin and Smit, *Chem. Weekblad*, 1919, **16**, 1210.

<sup>12</sup> Fouquet, *Compt. rend.*, 1910, **150**, 280.

<sup>13</sup> Naveau, *Sucre Belge*, 1943, **62**, 310, 336.

<sup>14</sup> Capelle, *Sucre Belge*, 1943, **62**, 335.

able<sup>15</sup> or crystallization sets in.<sup>16</sup> This limit is equivalent to a supersaturation of 2.0 at the usual observation temperature of 25° C, but may be increased slightly to about 2.4 by cooling to -10° C. This temperature is a lower limit set by the ice-sucrose eutectic point.

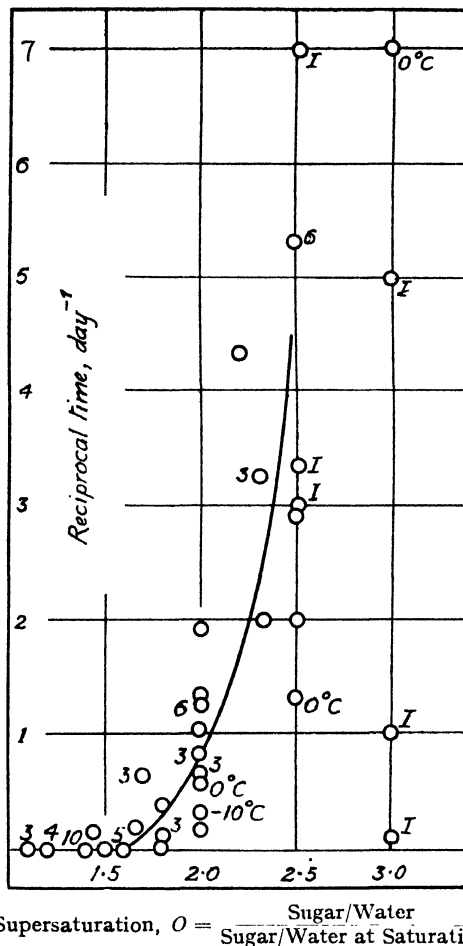


FIG. 1.—Time of appearance of crystals in sucrose syrups prepared by vacuum evaporation or active boiling, and curing at 20° C above saturation. Points are individual samples at 25° C unless designated as the average of several samples, or other temperatures. *I* signifies partly degraded solutions.

The times at which the beginning of crystallization was observed in solutions prepared in this way are presented in Fig. 1. The usual observation temperature was 25° C, others being properly designated on the Figure. The tubes were rotated slowly and the usual sample was about 10 g. solution. Inversion, by test, and/or degradation, by colour, was apparent in solutions above  $O = 2.0$ . None the less, observations were made in this higher range, and while some results suggest a monotonous extension of

<sup>15</sup> Montgomery and Wiggins, *J. Soc. Chem. Ind.*, 1947, **66**, 31.

<sup>16</sup> Stare, *Chem. Zlb.*, 1940, **2**, 2826; *A.C.S. Abstr.*, **36**, 6033.

the curve, others indicate a radical change in its nature. It is considered, however, that this change is caused by the impurities present; for addition of invert sugar, caramel or degraded syrups to pure syrups at lower concentrations, greatly prolongs the time required for crystallization.

The data suggest the stability of solutions less than about 1.6 supersaturated,<sup>17</sup> and the very rapid onset of nucleation above this concentration. The beginning of crystallization in syrups, prepared in the ordinary manner may be represented empirically by equations of the form:

$$(O - a)t = \text{const.},$$

where  $O$  is the supersaturation,  $t$  the time and  $a$  a constant:  $a$  was evaluated as 1.0, 1.2 and 1.05 in three cases surveyed, and 1.37 from some of Waterman and Gentil's<sup>2</sup> data. These are tantamount, of course, to Ostwald's metastable limit.

**Effect of Stirring.**—Increased rate of rotation of the tubes had no appreciable effect on the observed nucleation times. Neither did glass propeller stirring, under oil, up to 300 rev./min. and below  $O = 1.4$ . Above this concentration, however, the nucleation times were greatly reduced the higher the concentration and faster the stirring. For instance, at  $O = 1.4$ , stirring at 300 rev./min. for two days did not especially encourage crystallization. At 100 rev./min. a 1.5 supersaturated syrup crystallized in  $4\frac{1}{2}$  hr., whereas without stirring or with gentle rotation it is normally stable for weeks. At  $O = 2.0$ , where unstirred solutions take about a day to develop a visible crystal, a cloud shows up within a few hours at 100 rev./min. and in about an hour at 300 rev./min. Any accidental contact of the stirrer with the sides of the container, or with added glass beads, induces crystallization very promptly, even at low supersaturations. The foreign, suspended material of ordinary refined sugar seems to have no appreciable effect upon the nucleation time, provided the curing treatment is sufficient.

These irregular results with stirring suggest the influence of viscosity; which factor, therefore, was investigated by means of the temperature coefficient of reaction. Three tubes in a set, at a constant supersaturation of 2.0 with respect to 0°, 25° and 40° C, were rotated slowly. The times of nucleation noted were remarkably uniform. If the rate of nucleation is taken to be inversely proportional to the time, and the energy of activation assumed constant between each pair of temperatures, the following activation energies are computed.

TABLE I  
TIME OF NUCLEATION AND ENERGY OF ACTIVATION, AT  $O = 2.0$

Temp. ° C	Time (hr.)	$E_{\text{Act.}}$ (kcal./mole)	$E_{\text{Act.}}$ for growth <sup>18</sup> (kcal./mole)
0	40, 43, 45 } 16, 18, 20 } 6, 7, 8 }	5.3 10.6	24.4 11.7
25			
40			

**Effects of Surface-active Agents.**—The addition of surface-active materials in minute amounts had no significant effect, contrary to expectations from discussion in the literature.<sup>3 13 14</sup> The action of Aerosol OT (octyl sodium

<sup>17</sup> This is equivalent to a supercooling of about 50°, which is somewhat larger than those reported for many melts and solutions; Van Hook, *Annual Tables of Physical Constants* (Princeton, N.J.) (in progress).

<sup>18</sup> Van Hook, *Ind. Eng. Chem.*, 1945, **37**, 782.

sulphosuccinate), which is summarized in Table II, is typical of the many different types which were studied. The absence of any marked effect confirms our earlier experience<sup>19</sup> that these agents alter neither the nucleation nor growth kinetics of sucrose solutions. However, when nucleation does occur in their presence it is much more prolific than otherwise.<sup>20</sup>

TABLE II  
EFFECT OF AEROSOL OT ON THE TIME OF NUCLEATION OF SUCROSE SOLUTIONS

Supersat. (25°)	0 % Aerosol			0.05 %			0.2 %		
	I. Appear- ance	II. Surface Tension (dyne/cm.)	III. Time	I.	II.	III.	I.	II.	III.
2.3	Clear	(85)	8½ hr.	Cloudy	(80)	6.4	Cloudy	(63)	7.8
1.7	"	81	34	"	35	48	"	29	41
1.4	"	80	56, > 3 wk.	"	30	> 3 wk.	"	29	> 3 wk.
1.2	"	80	∞	Clear	30	∞	"	30	∞
1.1	"	79	∞	"	30	∞	"	29	∞

### Discussion

The times reported represent the sum of the time required to establish at least one stable nucleus, and the time for this embryo to grow to visible size. There is likewise the disturbance involved in the transfer from the curing temperature to that of the bath. The growth time is undoubtedly short at all but very small supersaturations<sup>2,18</sup>; while the transfer factor is common to all observations and will only alter the position of the curve and not its nature.<sup>21,22</sup> This shift cannot be appreciable in the present instance, since essentially the same results are obtained under various treatments.

The performance reported here is definitely contrary to the homogeneous theory of nucleation as espoused by Tammann and his school.<sup>10</sup> Any straightforward heterogeneous theory,<sup>6</sup> in the sense of foreign nuclei,<sup>4,5</sup> likewise seems inapplicable; since variable curing (provided this is at least 20° above the saturation point, yet not so severe as to hydrolyze or degrade the sugar) has no effect upon the observations. It seems quite clear that those concentration fluctuations which form at least critical-size nuclei are the origin of the crystallization observed in these experiments. The Volmer theory<sup>7</sup> for condensed systems is adequate to explain the results.<sup>3</sup>

This theory, as modified by the influence of viscosity,<sup>23</sup> and extended by the absolute reaction rate theory,<sup>24</sup> suggests that the rate of nucleation is

$$\dot{N} = \alpha \frac{NkT}{h} \exp[-(\Delta F^* + \Delta F_{\text{Visc.}}^*)]/kT,$$

where  $\Delta F^*$  is the free energy of activation involved in forming the nucleus,

<sup>18</sup> Bruno, M.S. Thesis (Holy Cross College, 1947).

<sup>20</sup> Van Hook, *Ring Surface Tensions* (in preparation). Highly concentrated sucrose solutions apparently salt out even traces of most of the surface-active materials investigated.

<sup>21</sup> Othmer, *Z. anorg. Chem.*, **91**, 209.

<sup>22</sup> Hammer, *Ann. Physik*, **33**, 445.

<sup>23</sup> Becker, *Ann. Physik*, 1938, **32**, 128.

<sup>24</sup> Turnbull, *J. Chem. Physics*, 1949, **17**, 71.



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$\Delta F_{\text{visc.}}^*$  the free energy of activation of viscosity, and  $x$  the mol fraction of solute.

The net free energy required to form the nucleus is also

$$\Delta F = \Delta F_s - \Delta F_v,$$

where  $\Delta F_s$  is the free energy required to form the surface, and  $\Delta F_v$  that gained in forming the mass of the crystal without any surface. Gibbs has shown that

$$\Delta F_s = (3/2)\Delta F_v;$$

whence

$$\Delta F = \frac{1}{2}\Delta F_v.$$

If these reversible values are identified with the energies of activation of the respective processes, we have

$$\Delta E_{\text{nucleation}} = \frac{1}{2}\Delta E_v.$$

The energy of activation of viscosity is approximately 1/3 that of growth,<sup>18</sup> so that as a crude approximation

$$\Delta E_{\text{nucleation}} = (5/6)\Delta E_{\text{growth}}.$$

This relative order of magnitude has been pointed out before in an empirical way.<sup>18</sup>

The Thomsen equation,

$$\ln(c/c_\infty) = \frac{2\sigma M}{RT} \frac{1}{r} \frac{1}{d},$$

with  $\Delta F = (1/3)\Delta F_s = (1/3)\sigma A = (4/3)\pi\sigma r^2$  (as spheres), suggests

$$\Delta F \sim \sigma^3/T^2$$

at constant supersaturation. In these expressions  $c$  and  $c_\infty$  are the solubilities at particle radii  $r$  and  $\infty$  respectively,  $\sigma$  the interfacial tension,  $A$  the surface, and  $M/d$  the molar volume. Since the activation energy is observed to increase with rising temperature at fixed interfacial conditions, it seems likely that some factor other than the work of forming the nucleus is involved in the nucleation process. Nothing definite is yet known about the entropy changes concerned in the above approximation, but the marked influence of stirring upon the rate of nucleation at higher concentrations is very suggestive of the viscosity as this factor.

**Surface Tension.**—The interfacial tension, which is so prominent in most crystallization theories, has received special attention in the case of sucrose solutions.<sup>3 13 14</sup> Since this interfacial tension between a solid and a liquid is difficult to evaluate, it has frequently been correlated with the ordinary surface tension of the liquid, although it seems questionable to specify it in this way. Dupré's rule for this type of interface is

$$\sigma_{sl} = \sigma_{sg} - \sigma_{lg} \cos \theta,$$

where  $s$ ,  $l$  and  $g$  indicate solid, liquid and gas phases respectively, and  $\theta$  is the contact angle of wetting of the solid. If the wetting is complete, and the surface tension of the solid is constant, we have

$$\sigma_{sl} = \sigma_{sg} - \sigma_{lg},$$

and

$$d\sigma_{sl} = -d\sigma_{lg}.$$

The former is Antonoff's rule for this case, and the latter indicates that ordinary surface-active materials, which usually decrease the liquid surface tension, may actually increase the interfacial tension at the solid surface.

It was found impossible to increase the surface tension of crystallizable sucrose solutions to any extent by additives; but ordinary wetting agents diminish it considerably. Even so, no great influence on the crystallization

time was observed, which is contrary to several reports in the literature under similar circumstances.<sup>3 18 18 25</sup>

A twofold oversaturated solution of sucrose in 68 % alcohol, whose surface tension was 26 dynes/cm., did not display crystals for 8 days, compared to about 1 day for an aqueous solution of the same supersaturation. Whether this prolongation is the result of lowered surface tension (and therefore possibly increased interfacial tension) or change in environment is not yet evident. These matters are being investigated further in this laboratory.

**Practical Implications.**—The extreme difficulty of preparing and preserving supersaturated sucrose solutions would augur well for the applicability of the heterogeneous theory, in spite of the greater significance of the thermodynamic theory. Under conditions which prevail in the sugar house, as well as in ordinary laboratory work, nucleation undoubtedly occurs by chance inoculation. Under these circumstances, it is merely the rate of growth to visible size which determines the observed nucleation time. Since this process has been shown to be unimolecular,<sup>26</sup> the observed equilateral hyperbola relation is an obvious one.

However, as the concentration increases, a very strong and abrupt influence of true nucleation sets in; thus accounting for the metastable limits usually reported.<sup>27</sup> Nucleation in condensed systems has all the attributes of a chain reaction,<sup>6 28</sup> which feature explains the autocatalytic "false grain" region<sup>27</sup> of the sugar boiler.

**Conclusions.**—The prominent features of the Volmer-Becker theory of nucleation are shown to be qualitatively applicable to supersaturated sucrose solutions. Quantitative aspects will be investigated.

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<sup>25</sup> Von Weimarn, *Z. Chem. Ind. Koll.*, 1907, **2**, 76; *A.C.S. Abstr.*, **3**, 393.

<sup>26</sup> Van Hook, *Ind. Eng. Chem.*, 1944, **36**, 1042.

<sup>27</sup> Webre, *Proc. 11th Conf. Assoc. tec. azucareros Cuba*, 197, p. 9.

<sup>28</sup> Langmuir, *Proc. Amer. Phil. Soc.*, 1948, **92**, 167.

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## THE RELATIVE RATES OF GROWTH OF STRAINED AND UNSTRAINED AMMONIUM NITRATE CRYSTALS

BY S. FORDHAM

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At a discussion on crystallization held in Bristol in February, 1948, it was suggested by Prof. N. F. Mott that crystals should grow more rapidly when strained than when unstrained. During the course of a more general programme of work on the crystallization of ammonium nitrate, an opportunity was taken of testing this suggestion.

### Experimental

The crystals of ammonium nitrate were prepared by slow evaporation at 26° C. It had been shown that Lissolamine A did not affect the crystallization

of ammonium nitrate under such conditions, so the vessels were treated before the experiment with a dilute solution of this compound to prevent "creep." Some of the crystals which developed were prisms with good faces of the {110} form and usually with {011} faces, although the latter were frequently poorly developed. Specimens about 1.5 mm. broad and 4-5 mm. long were selected, roughly dried, and then shaken with a solution of Fixanol C in carbon tetrachloride. The latter emulsified any adhering mother liquor and gave completely dry crystals, which were stored in a desiccator for use. Crystals were used within 24 hr. of preparation, during which time they retained quite adequate plasticity.

The dry crystals were measured by means of a low-power microscope, and for each experiment two batches of five crystals each were selected, so as to be as nearly as possible equivalent in size and shape. Complete similarity was not, of course, attainable but in most cases variations in linear dimensions were within 10 %. The crystals of one batch were strained by bending round a rod of 4 mm. diam., and their total weight found; the other batch was weighed without straining. The strained and unstrained crystals were arranged

TABLE I  
GROWTH OF STRAINED AND UNSTRAINED AMMONIUM NITRATE CRYSTALS

Original breadth mm.		Mean Growth mm.	Extra Growth mm.	Ratio of Extra to Mean Growth
Strained	Unstrained			
2.112	2.080	0.451	0.010	0.022
1.235	1.347	0.444	0.019	0.043
1.408	1.500	0.352	0.037	0.105
1.594	1.685	0.130	0.021	0.161
1.467	1.572	0.125	0.008	0.064
1.596	1.693	0.189	-0.001	-0.005
1.396	1.428	0.119	0.032	0.269
1.208	1.221	0.091	0.017	0.187
1.292	1.318	0.126	0.026	0.206
		Mean	0.019	0.117

Mean growth is the average linear growth of all crystals.

Extra growth is the amount by which the growth of strained crystals exceeded that of unstrained.

alternately in a crystallizing dish, and allowed to grow in an ammonium nitrate solution evaporating at 26° C. After the required time the crystals were dried as before and the batches re-weighed. In the earlier trials growth by 100 % in weight was reached, but in the later experiments this was reduced to 20-40 %, which was the minimum for which the experimental arrangements were suitable.

From the measured weights, the mean breadths before and after growth were calculated on the assumption that the crystals were rectangular parallelepipeds, the lengths of the sides being in the ratio 1 : 1 : 3. The increase in breadth was used as a measure of growth, and was in most cases greater for the strained crystals. The results are given in Table I. It will be seen that in all cases except one, the strained crystals grew more than the unstrained, but that the scatter of the recorded results was large. The standard deviation was calculated and the *t*-test applied to determine the significance of the mean, when it was found that the probability that the result was a chance variation from zero was about 0.002. It appears very likely, although not definitely proved, that strained crystals of ammonium nitrate grow faster than unstrained.

Local variations in rate of growth undoubtedly occurred in these experiments, although their effect should have been eliminated by the method of analyzing the results. An attempt was made, however, to attain more uniform conditions by growing the crystals in a vertical tube with an air current sufficiently strong to maintain agitation. The trial was discontinued because fresh nucleation was extensive and results were very erratic.

### Discussion

It appears most probable that ammonium nitrate grows faster from solution when strained. Such a statement, however, needs some elaboration before its true meaning becomes clear. The presence of strain should be shown at the surface by dislocations, and the increased rate of growth should be attributed to the presence of such dislocations and discontinuities. These experimental results do in fact support the theory that the normal growth of crystals is due to the propagation of dislocations.

It would be expected that as the strained crystals grow, the number of effective extra dislocations remaining in the surface would diminish, so that the rate of growth of the two types of crystal should gradually become equal.

There is, in fact, no significant correlation between the figures for "extra growth" and "mean growth" recorded in Table I, and it is concluded that the effect of the straining had been eliminated before the shortest experiment was complete. Indeed, a formal rate may be calculated by taking the ratio of extra to mean growth, as in the last column of Table I, and it is found to have a negative regression coefficient on the mean growth, with significance 0.05-0.1. It would appear therefore that the method of straining used in these experiments caused dislocations which did not persist through a fresh layer 0.05 mm. thick, but which made crystallization more rapid in the early stages by at least 20 % of its normal speed.

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## CRYSTAL GROWTH FROM SOLUTION

### I. Layer Formation on Crystal Faces

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In this and the following paper, the results of many observations and experiments made over a number of years (since 1932) are collected and discussed. The work on crystal growth from solution which has been carried out in this laboratory from time to time, as opportunity occurred, was started with the object of discovering how the rates of growth of crystal faces are related to the structure of the crystal face and the concentration of solute in the surrounding solution. It was at first hoped that the problem could be treated in terms of simple physico-chemical concepts: the rate of growth of a particular face was perhaps some function of the supersaturation of the solution in contact with the face, while different faces of the same crystal or of crystals of different substances would be characterized by different constants which would depend on the surface forces. Such relations have usually been assumed in previous theoretical speculations, such as those of Berthoud<sup>1</sup> and Valetton.<sup>2</sup> But it became evident that, on the one hand, there was no correlation between the rate of growth of a particular type of face and the supersaturation at the face ;

<sup>1</sup> Berthoud, *J. Chim. Physique*, 1912, **10**, 624.

<sup>2</sup> Valetton, *Z. Krist.*, 1924, **59**, 135, 335.

and on the other, that a growing crystal face is not a uniform surface, and that surface forces must vary at different points. The work then became frankly exploratory, and as far as possible uninfluenced by preconceived hypotheses. The surfaces of growing crystals of many substances were observed as closely as possible under the microscope, in order to learn as much as possible about the manner of deposition of solute and the fine structure of growing faces; this work is reported in the present paper. The other aspect of the problem—the concentration of solution and its variation round a growing crystal—is dealt with in the second paper.

The polyhedral habit of most crystals suggests that material is deposited on the faces in successive layers; and the theoretical work of Kossel,<sup>3</sup> Stranski<sup>4</sup> and Brandes and Volmer<sup>5</sup> supports the idea that, at any rate for ionic crystals of NaCl type of structure, this is the manner in which ions are built on to the crystal: the energy yield when an ion is added to an incomplete layer is greater than that for the starting of a new layer, and therefore there is a tendency for a layer, once started, to be completed rapidly, the inception of the next layer being delayed. Direct experimental evidence on the matter, on the ionic or molecular scale, is not available; but layers a few molecules thick have been detected on crystals of *m*-toluidine by Marcelin<sup>6</sup> and by Kowarski.<sup>7</sup> This substance grows from alcoholic solution in extremely thin plates, thin enough to give interference colours like those seen in oil films on water; discontinuities in the shade of interference colour, which meant discontinuities of thickness, could be seen moving across the crystal faces, and it was calculated that in some circumstances the layers thus revealed were only a few tens of ångström—in fact, only a few molecules—in thickness. Similar thin layers were observed by Volmer<sup>8</sup> on thin crystals of  $\text{PbI}_2$  formed by mixing solutions of  $\text{Pb}(\text{NO}_3)_2$  and KI. Not many substances grow in sufficiently thin plates to give this type of evidence; but we found that thick crystals of some substances, when observed at high power, using dark ground illumination, show layers spreading across the faces, and these, to be visible at all, must be very much thicker than those seen on *m*-toluidine or  $\text{PbI}_2$ ; they must be at least several hundred ångström thick. On a few crystals the layers are so thick that they can be seen either in ordinary transmitted light or in birefringent crystals by using crossed Nicols. It was possible to make many observations of these layers on a variety of different crystals, to observe their point of origin, to measure their thickness and rate of spreading, and the effect on them of the presence of dissolved impurities and of different solvents.

Most of the work has been qualitative, and constitutes an extensive superficial survey of the phenomenon of layer formation on crystals of many different substances. In all the experiments, a drop of warm saturated solution was placed on a warm microscope slide, covered with a thin cover-slip, and observed while cooling on the microscope stage. A cardioid condenser was used to give dark-ground illumination at high powers.

The outstanding generalizations which emerged from these observations are the following—

1. Layers very often start, not from edges or corners of crystals, but from the centres of faces, spreading outwards towards the edges.

<sup>3</sup> Kossel, *Nach. Ges. Wiss. Göttingen*, 1927, 135; *Metallwirtschaft.*, 1929, 8, 877; *Naturwiss.*, 1930, 18, 901.

<sup>4</sup> Stranski, *Z. physik. Chem.*, 1928, 136, 259.

<sup>5</sup> Brandes and Volmer, *Z. physik. Chem. A*, 1931, 155, 466.

<sup>6</sup> Marcelin, *Ann. Physique*, 1918, 10, 185.

<sup>7</sup> Kowarski, *J. Chim. Physique*, 1935, 32, 303, 395, 469.

<sup>8</sup> Volmer, *Z. physik. Chem.*, 1923, 102, 267.



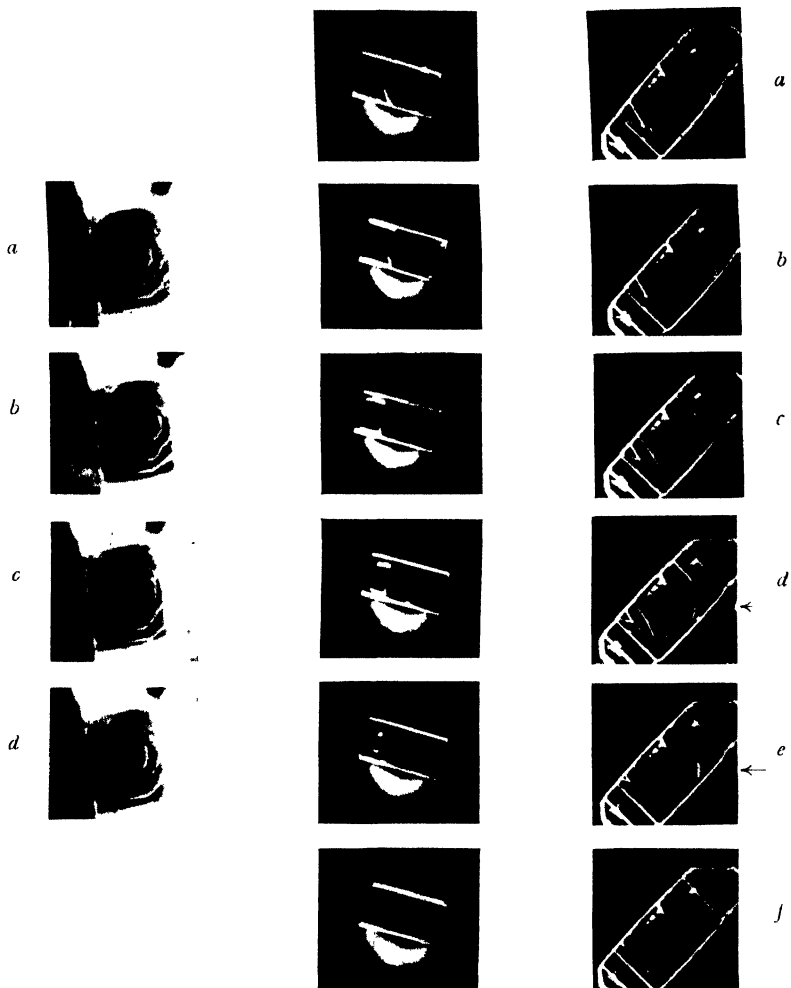


FIG. 1.

FIG. 2.

FIG. 3.

FIG. 1.—NaCl. Photographs at 1-sec. intervals. ( $\times 300$ )

FIG. 2.—NaCl.  $\frac{1}{2}$ -sec. intervals. Pit formed by layer encirclement, and subsequently filled in. ( $\times 245$ )

FIG. 3.— $\text{KH}_2\text{PO}_4$ . 1-sec. intervals. ( $\times 35$ )

2. The thickness of the layers on many crystals increases as the layers approach the edges of the crystal faces.
3. The boundaries of the layers are often irregular, especially when growth is rapid; but as growth slows down there is a tendency to regularity of shape, the actual shape conforming to the symmetry of the crystal face.
4. Dissolved impurities may strongly influence the thickness and the shape of the layers, the effect being highly specific.
5. Thick layers have been seen only on crystals of certain ionic or polar substances; they have not been seen, under the experimental conditions used, on crystals of non-polar substances.

### Experimental

**Sodium Chloride.**—The first four of the above generalizations are well illustrated by the phenomena observed in experiments with sodium chloride. The cubic crystals usually lie on a microscope slide on a cube face, so that one face is seen normally and four others edgewise. On the face seen normally, layers were usually observed to be spreading outwards from a point roughly in the centre of the face. It was not possible to locate the point exactly; nothing could be seen at the centre of the system of layers, and the layers only became visible at a distance from the centre which varied considerably but was often one-quarter to one-half of the distance to the edge. From the increasing plainness of the layer boundaries towards the edges of a face, it appeared that the layers increased in thickness as they spread outwards. The layers were usually rather irregular in shape, though sometimes suggesting a square; but the addition of 1 %  $\text{CaCl}_2$  to the solution had the effect of making the layers more regular in shape, roughly octagonal, and more easily visible. It was possible to obtain cinematograph records of the growth of the layers under these circumstances. (A Pathe camera (9.5 mm. film), with the lens removed, was used at normal speed. The microscope objective (2.9 mm. oil immersion) cast an image straight on to the film. For further details see paper by Emmett.<sup>9</sup>) Four shots selected from the cinematograph film are shown in Fig. 1 *a-d*; they show the same face at successive intervals of 1 second. A new layer, which is barely visible in 1 *b*, is easily visible in 1 *c* and has spread considerably in 1 *d*. On the faces seen edgewise, it was sometimes possible to see the thickness of the layers, but in general, in the case of sodium chloride, the layers were so thin that they showed up best on the face seen normally, owing to the scattering light by the edge of each layer. (By ordinary transmitted light the layers could not be seen at all.)

The average thickness of the layers was measured by first focusing on a particular point on the surface, counting the passage of some 30–50 layers past the point, refocusing and reading off the change in position of the calibrated fine adjustment screw of the microscope. After correcting for the refractive index of the solution, the total thickness of the layers was obtained, and hence the average thickness of one layer. In several experiments, the layer thickness was found to be 2800, 3500, 4100, 3600 and 1700 Å. As the length of the unit cell edge of sodium chloride is 5.63 Å, each layer edge is a wall 300–700 unit cells high (or twice this number of atoms).

The rate of spreading of the layers in one experiment was found by measuring a cinematograph film. All the layers spread at about the same uniform speed, the rate of advance of an edge (in the direction of a cube edge) being  $2.5 \times 10^{-4}$  cm./sec.; the rate declined a little as time went on. (The rate of spreading, like the thickness, varied considerably with the specimen of salt and the conditions of the experiment, and this figure by itself has no great significance; it is quoted simply to give an idea of the order of magnitude.) The rate of spreading of a layer diminishes slightly as it spreads outwards.

Certain impurities affected the growth of the layers quite profoundly. The effect of  $\text{CaCl}_2$  in making the layers thicker and more regular has already been

<sup>9</sup> Emmett, *J. Micro. Soc.*, 1943, 63, 26.



mentioned;  $\text{Na}_3\text{SbO}_3$  had a similar effect; but most other substances which were tried either had the reverse effect—that is, they made the layers thinner—or else had no marked effect. The following substances had the effect of making the layers thinner; and, if added in sufficient concentration, gave NaCl crystals which showed no sign of layers. The percentage figure given is the concentration required to give clear crystals showing no layers:

$\text{Pb}(\text{NO}_3)_2$  (0.05 %),  $\text{PbCl}_2$  (0.05 %),  $\text{Bi}(\text{NO}_3)_3$  (0.1 %),  $\text{BiCl}_3$  (0.1 %),  $\text{MnCl}_2$  (2.0 %),  $\text{CdCl}_2$  (0.02 %),  $\text{SrCl}_2$  (2.0 %),  $\text{SnCl}_2$  ( $\sim 2$  %).

The following substances appeared to have no appreciable effect—

$\text{HCl}$ ,  $\text{Na}_2\text{SO}_4$ ,  $\text{NiCl}_2$ ,  $\text{CoCl}_2$ ,  $\text{SbCl}_3$ ,  $\text{Al}(\text{NO}_3)_3$ ,  $\text{BaCl}_2$ ,  $\text{MgCl}_2$ ,  $\text{ZnCl}_2$ ,  $\text{FeCl}_3$ ,  $\text{LiCl}$ ,  $\text{PtCl}_4$ ,  $\text{HgCl}_2$ , and cerous nitrate. The effect of impurities on the formation of layers is thus specific, and recalls the modification of crystal shape by dissolved impurities, which is also a highly specific effect. But note that the only substances in the above list which caused the appearance of other than cube faces were  $\text{Bi}(\text{NO}_3)_3$  and  $\text{BiCl}_3$ , which produced (110) as well as (100) faces.

In the great majority of experiments, only one system of layers was seen on any one face; but on a few occasions two systems, spreading from different points, were seen; and on one occasion, when an unusually large crystal was observed, no less than five systems were seen. In all cases the points from which the layers spread (whether there was one centre or more on a face) were not on the corners or edges, but well within the boundaries of the face; and when there was only one point, it was roughly at the centre of the face.

Another phenomenon seen occasionally was the formation of a pit by encirclement of a small area by a layer growing all round it. This is well shown by the series of photographs in Fig. 2, in which a pit forms towards each end of a long rectangular face on which a layer system is spreading from the centre; these pits are soon filled in. On this occasion the process of formation and subsequent filling-in of a pit was repeated several times in rapid succession. The phenomenon recalls the triangular pits on diamond crystals reported by Tolansky and Wilcock<sup>10</sup> and believed to have arisen during growth; the actual growth of diamond cannot be observed, but the present example (and many others which have been seen on various crystals) shows that layer encirclement does occur and lends support to Tolansky and Wilcock's explanation.

**Cadmium Iodide.**—This substance grows in the form of hexagonal plates; if the basal planes are observed when the crystals are growing from aqueous solution, layers can be seen spreading over the faces, even in ordinary transmitted light. The layers observed in these experiments were roughly circular in shape, or occasionally vaguely hexagonal. Fig. 4 shows a typical crystal with a single system of layers spreading outwards from a point which can be located more precisely than in the case of sodium chloride; the layers can be seen much nearer to the origin. Fig. 5 shows an example in which there are two origins, and the layers are more nearly hexagonal in shape.

The layers on this substance were usually a little thicker than those on sodium chloride—from 3000 to 5000 Å; the reason why they are often more easily visible than those on sodium chloride is partly that they are thicker, but chiefly because of the high refractive index of  $\text{CdI}_2$ . The rate of spreading was about one-fifth that of sodium chloride.

**Potassium Dihydrogen Phosphate.**—This is another substance on which layers can often be seen without the aid of dark ground illumination. It is tetragonal, and grows in the form of prisms of square cross-section terminated by pyramids, and the crystals usually lie on the microscope slide so that the long rectangular (110) prism faces are seen normally. On these faces, layers can be seen spreading outwards from a point which is, more often than not, roughly in the centre of the face. The layers are sometimes rectangular, as in Fig. 6, but are more often very irregular in shape. A phenomenon which this substance shows particularly clearly is that of very thin rapidly advancing layers overtaking thicker and more slowly advancing ones; this appears to be the way in which thick layers are built up, and if we may imagine the same

<sup>10</sup> Tolansky and Wilcock, *Nature*, 1946, **157**, 583.



FIG. 4.

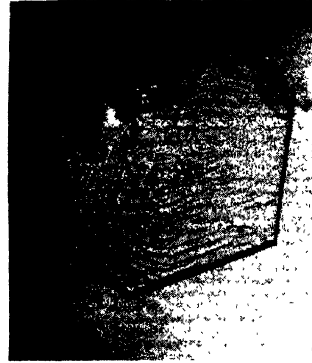


FIG. 5.

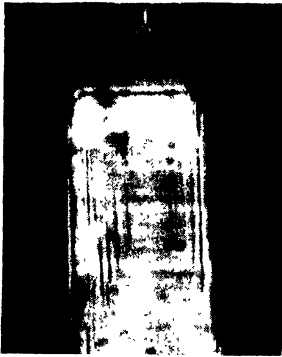


FIG. 6.



FIG. 7.

FIG. 4 and 5.— $\text{CdI}_2$ . ( $\times 190$ )

FIG. 6 and 7.— $\text{KH}_2\text{PO}_4$ . Polarized light, crossed Nicols. ( $\times 150$ )



process occurring on a smaller scale, it is probably in this way that layers of visible thickness are built up from original layers which are perhaps one ion or a few ions thick. The increasing thickness of layers as they grow outwards is also shown strikingly by this substance; note that in Fig. 3 no layers can be seen at the centre, but they become very plain towards the ends of the crystal; when the cinematograph film is watched, each layer gradually becomes visible as it grows outwards, and this is probably due to the overtaking of much thinner layers, too thin to be seen. Another phenomenon which occurs in Fig. 3 is the formation of a pit by encirclement of a small area by a layer growing all round it. The beginning of encirclement is seen on the right-hand side of 3 *d* (see arrow); the pit is clearly visible in 3 *e* (arrow); at 3 *f* it has disappeared, having been filled in by inward growth of the layer. One more observation on Fig. 3: the confused appearance of the bottom left-hand corner of each photograph is due to the existence of a number of irregular layers, some of which overtake others; the sequence of events can only be properly appreciated by watching the actual process or the cinematograph record.

The addition of phosphoric acid to the solution had the effect of making the layers thinner and more difficult to see; when  $K_2HPO_4$  was added to the solution no layers could be seen at all.

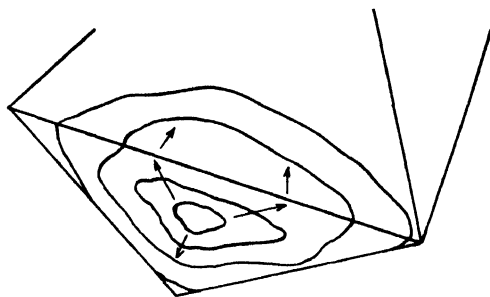


FIG. 8.—Layers "wrapping over" from one face to another. (Octahedral faces of  $Pb(NO_3)_2$ .)

On crystals of this substance, the layers are often well seen when crossed Nicols are used; the interference colour arising from the birefringence of the crystal varies in shade according to the thickness, and the colour effects show up the growth of the layers in a beautiful manner, though appearances are sometimes confused, owing to the fact that two systems of layers, one above and one below the crystal, are seen simultaneously. Some very striking cinematograph shots (in colour) were secured under these conditions. Fig. 7 is a monochrome "still" from one of these films; it shows, in the upper part, numerous thin irregular-shaped layers, and in the lower part, some more regular formations. This photograph also shows other striking characteristics of this substance, when grown rapidly on a microscope slide—the tendency for different parts of the same rod-like crystal to grow almost independently (note the contrast between the two layer systems in the two halves), and the tendency to form very thick sheath-like layers, embracing all four sides of the tetragonal prism, which grow slowly along the prism.

**Other Salts.**—Many other salts were observed, and in the majority of cases no layers were seen; but several other crystals were found to exhibit visible layers. Among them was lead nitrate, the normal habit of which is octahedral. The layers which grew on the triangular faces were roughly triangular in shape, when they grew from a point in the centre of a face. Sometimes, on the other hand, layers seemed to be spreading from a corner or an edge; the origin of those spreading from an edge was, perhaps, indicated by occasional observations that a layer, on reaching an edge, wrapped over on to the next face, as in Fig. 8. The addition of Methylene Blue to the solution caused the crystals to grow as cubes; on these also layers were seen, usually apparently growing

from corners and edges ; but whether the corners and edges were the real origin of the layers is doubtful, in view of the " wrapping-over " effect just mentioned. When a layer system can be seen spreading from the centre of a face, there is no doubt about the origin, but when it appears to come from an edge or corner, it may be a legacy from another face, which cannot be examined. The addition of sodium nitrate to the solution had no effect, but when nitric acid was added no layers could be seen. When both sodium nitrate and nitric acid were added layers were again seen.

Sodium nitrate, growing from pure solution, showed no layers, but the addition of lead nitrate to the solution caused layers to appear. No other nitrates had any effect when added to  $\text{NaNO}_2$  solution ; those of Ca, Sr, Ba, Cu, Ni, Ag, Hg, Bi and Al as well as nitric acid were tried.

Potassium sulphate and potassium chromate showed extremely thin layers. The addition of  $\text{H}_2\text{SO}_4$ , NaOH, KOH, or  $\text{Na}_2\text{SO}_4$  to a solution of potassium sulphate did not affect the formation of layers. Alum showed slight indications of layers.  $\text{FeSO}_4 \cdot (\text{NH}_4)_2 \text{SO}_4 \cdot 6\text{H}_2\text{O}$  showed clearly defined layers, and it was on one of these crystals that a system of layers was seen growing inwards (filling a pit), on the same face with a normal outward-growing system (see Fig. 9) ; the inward-growing system could be seen to be due partly to the wrapping-over of layers from contiguous faces.

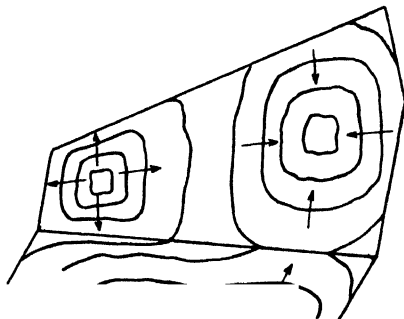


FIG. 9.—Outward-growing and inward-growing layer systems on the same face of a crystal of ferrous ammonium sulphate. Note the part played by the " wrapping-over " effect.

Potassium iodide showed some confused appearances which might be interpreted as layer formation ; these were seen near edges and corners, but the direction of spreading was not clear. Mercuric chloride showed definite layers ; so did magnesium nitrate.

Sodium formate, sodium diethyldithiocarbamate and sodium phthalate showed very thin layers.

Layers could not be observed on any of the following crystals— $\text{NaClO}_3$ ,  $\text{NaIO}_3$ ,  $\text{NaF}$ ,  $\text{NaBrO}_3$ ,  $\text{NaNO}_3$ ,  $\text{NaNO}_2$ , sodium antimonate and sodium nitroprusside ;  $\text{KNO}_3$ ,  $\text{KNO}_2$ ,  $\text{KClO}_3$ ,  $\text{K}_2\text{Cr}_2\text{O}_7$ ,  $\text{KSCN}$ ,  $\text{KClO}_4$ ,  $\text{K}_2\text{S}_2\text{O}_8$  ; sodium citrate, potassium oxalate and tetraoxalate ; ammonium chloride, either from pure solution or a solution containing urea ;  $\text{LiCl}$ ,  $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ ,  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ ,  $(\text{NH}_4)_2\text{SO}_4$ , ammonium vanadate, chrome alum ; and  $\text{NaH}_2\text{N}_2\text{H}_4\text{PO}_4 \cdot 4\text{H}_2\text{O}$ .

All those substances on which layers were seen have moderate or high solubilities (the lowest being  $\text{HgCl}_2$  with a solubility of about 6 % at  $20^\circ\text{C}$ ). The following slightly soluble substances were observed, but no layers were seen on any of them—

$\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$ ,  $\text{CaCl}_2$ ,  $\text{Ca}(\text{OH})_2 \cdot \text{H}_2\text{O}$ ,  $2\text{Na}_3\text{PO}_4 \cdot \text{NaF} \cdot 19\text{H}_2\text{O}$ ,  $\text{Na}_4\text{B}_2\text{O}_7 \cdot 10\text{H}_2\text{O}$ ,  $\text{PbCl}_2$ ,  $\text{AgSO}_4$ ,  $\text{Li}_2\text{CO}_3$ ,  $\text{Sr}(\text{OH})_2$ ,  $\text{Ba}(\text{OH})_2$ ,  $\text{KClO}_4$ .

**Organic Substances.**—The following crystals have been observed, but no layers have ever been seen under the conditions of these experiments—

Cane sugar, hydroquinone, propionamide, phthalic acid, citric acid (in water), naphthalene, benzophenone, camphor, benzil, stilbene, *p*-nitrophenol, methylglyoxime, dimidone, dichlorobenzene, acetanilide and  $\alpha$ -naphthol (in alcohol) ; and anthracene in benzene solution.

On the other hand, urea, acetamide, pyrocatechol and chloramine-T ( $\text{CH}_3\cdot\text{C}_6\text{H}_4\text{SO}_2\text{Na}\cdot\text{HCl}\cdot 3\text{H}_2\text{O}$ ) showed definite layers, when grown from aqueous solution; so also did sodium formate, sodium diethyldithiocarbamate and sodium phthalate. Note that all the substances on which layers were seen are either ionic or else contain strongly polar groups.

**Influence of Solvent.**—It appeared from all the foregoing observations that layers thick enough to be seen by ordinary light under the conditions of these experiments are only formed on crystals which contain either ions or strongly polar groups; in view of this apparent influence of electrostatic forces, it seemed of interest to study the growth of crystals in solvents of lower dielectric constant than water. Several substances which had been observed to give visible layers when grown from aqueous solution, and which are also soluble in ethyl alcohol, were therefore crystallized from this solvent. These substances were cadmium iodide, potassium dihydrogen phosphate, sodium formate, sodium diethyldithiocarbamate, urea, acetamide, pyrocatechol and chloramine-T. In all cases, layers did not appear on crystals growing from alcoholic solution. In three cases—urea, potassium dihydrogen phosphate and cadmium iodide—alcohol-water mixtures were also used, and it was evident that the layers became thinner with increasing proportion of alcohol. When urea was grown from methyl alcohol layers were seen, as in water solution; but cadmium iodide in methyl alcohol showed no layers. It seems reasonable to attribute these effects to the fact that the dielectric constant of ethyl alcohol (26 at 20° C) is much lower than that of water (81 at 18° C); the dielectric constant of methyl alcohol (31 at 20° C) is a little higher than that of ethyl alcohol.

### Discussion

**Formation of Thick Layers.**—It seems likely that crystals in general (at any rate, those with definite faces) grow by the spreading of discrete layers one after another across the faces. On many crystals these layers are too thin to be seen by visible light; but, as we have seen, on quite a number of crystals the layers are sufficiently thick to be seen either by dark ground illumination or sometimes even by ordinary transmitted light or between crossed Nicols; these layers are often some thousands of ångström in thickness. That the layer-spreading process occurs on the much smaller scale of a few molecules has been shown by the observations of Marcelin<sup>6</sup> and Kowarski<sup>7</sup> on *p*-toluidine, which, because it grows as exceedingly thin plates, permits observations by a method sensitive to much smaller differences of thickness than those revealed under the present conditions; and the process of formation of thick layers from thin ones is demonstrated by our frequent observations that thin layers, spreading more rapidly than thicker ones, overtake underlying thicker layers and add to their thickness.

Why are thick layers, hundreds of ions or molecules in thickness, built up? Why do not the thin primary layers, which are perhaps one ion or molecule, or a few ions or molecules, in thickness, proceed independently?

It might be urged that thin layers spread faster than thick ones, simply because less solute is required to extend a thin layer a certain distance than to extend a thick layer to the same distance. But this is beside the point: a system of thin layers of a certain height needs precisely the same amount of solute to spread a certain distance as a system of fewer thick layers of the same total height. What we have to explain is the tendency of a system of a large number of thin layers to break up into a system of a few thick layers. Although no quantitative theory based on a consideration of surface forces can be offered, we can at any rate link up the phenomenon of thick layer formation with the general principles of crystal morphology. When a succession of thin layers, of ionic or molecular thickness, is spreading across a crystal face, the surface (see Fig. 10 *a*) is not a face of low indices but a face having very high indices. Now the outstanding generalization

of crystal morphology is the universal tendency for the bounding surfaces to be faces of low indices; when growth comes to an end the faces are found to be those of low indices, often indeed the simplest possible indices; these are the faces which are either parallel to, or are simply related to, the edges of the unit cell. Faces with high indices, if they are artificially created by cutting or dissolving a crystal, eliminate themselves because their rates of growth (thickness deposited on the surface in unit time) are higher than those of simple faces. The elimination may occur by apparently straightforward growth as in Fig. 11 *a* or by a process of breaking-up into steps as in Fig. 11 *b*. Faces of high indices are thus less

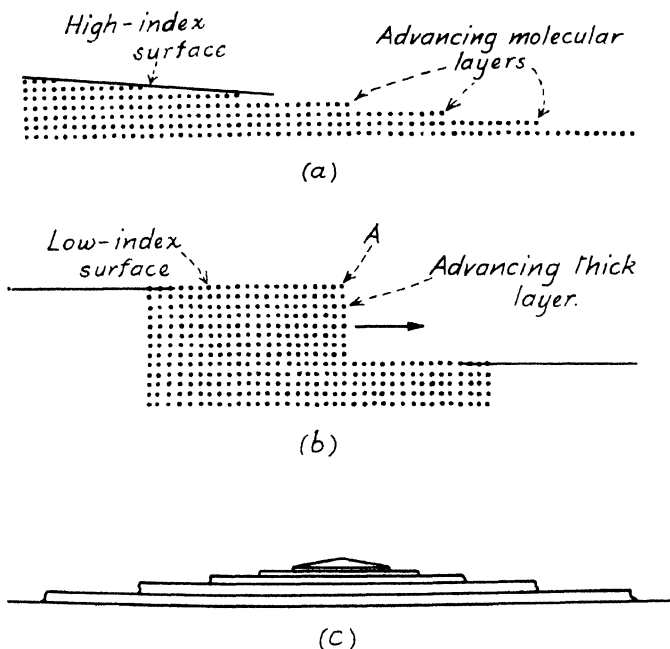


FIG. 10 (a) A system of molecular layers spreading, one after another, across a face constitutes a high-index surface.

(b) The break-up into large steps has the effect of making the major part of the surface a low-index surface.

(c) Idealized representation of the surface of a growing crystal. (Heights of steps much exaggerated.)

stable as surfaces, and presumably have a higher surface energy, than those of low indices. There will therefore be a tendency for the high-index faces created by the system of spreading layers (Fig. 10 *a*) to break up into comparatively large steps, so that the bounding surfaces have low indices (Fig. 10 *b*). (Actually the edge of a step is usually a high-index surface (see below); nevertheless, the break-up into large steps does have the effect of making the great majority of the surface (the tops of the layers) a low-index surface.) There is, of course, no sharp distinction between the original surface which can appropriately be regarded as a high-index face (Fig. 10 *a*) and the surface with large steps (Fig. 10 *b*) which is predominantly a low-index surface; there is a continuous change of surface energy with step-height. Thus the reason why thin layers spread more

rapidly than thicker ones, and therefore overtake thicker ones, is in all probability that there is a greater surface energy at a low step than at a high one. The formation of thick layers hundreds of ions or molecules thick is thus seen to be just another manifestation of the great morphological principle of simple indices.

The "vicinal" faces which have been found on minerals and on crystals grown in the laboratory probably consist of systems of layers having a step-height smaller than the wavelength of light; the crystals exhibiting vicinal faces are probably crystals whose growth has not gradually slowed down with time, but has for some reason been arrested. The existence of vicinal faces on growing crystals was shown by Miers (1903), who also observed that the inclination varied during growth. The crystals he studied were alum, sodium chlorate,  $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$  and  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ ; all these have been studied in the present work, and only on alum have any indications of layers been seen; but if our interpretation of vicinal faces is correct, Miers' observations may be taken as evidence that layers do form on the other two crystals but are too thin to be seen.

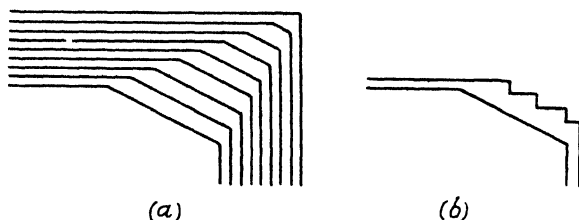


FIG. 11.—Elimination of high-index surface, (a) by apparently straightforward growth (the rate of growth of the high-index face being greater than those of neighbouring low-index faces), (b) by step formation.

The layer thickness built up on any crystal presumably depends on the difference between the surface energies of high-index and low-index faces (in relation to the surrounding solution), which may perhaps be formulated for the present circumstances as the change of surface energy with step-height; our observations have shown that this is different for each crystal, and, moreover, is strongly influenced by specific impurities in the solution. The only generalization we can make is that since thick layers were never seen on crystals of non-polar substances, but were seen on a number of crystals containing ions or polar molecules, the change of surface energy with step-height is steeper in ionic or polar crystals than in non-polar ones. It is entirely reasonable that this should be so; in a crystal composed of ions or polar molecules, different faces present very different arrangements of positive and negative charges, and the surface forces would be expected to vary sharply with the distribution of surface charges; but in non-polar molecular crystals there are no sharp electrostatic differences, and it is to be expected that the differences between the surface forces of different faces would be less marked. The differences between various ionic and polar crystals, some of which show thick layers, while others do not, remain unexplained; we have not been able to detect any correlation between chemical constitution and the presence or absence of thick layers. In view of the powerful influence of dissolved impurities, any relationship could only be expected to be found if highly purified substances were used.

The manner of deposition on the edge of a layer must also be considered. A layer edge several hundred atoms high is quite a large face, from the atomic point of view; does deposition occur on it by the formation of surface



nuclei and the subsequent spreading of layers on a smaller scale than that involved in the thick layers, or does solute pile on in a more indiscriminate manner? And if layers are formed, where do they start? The answer to this question is probably bound up with the question of the nature of the surface of a layer edge. Most growing layers are irregular in shape and even if (on a cubic crystal) the edges are at right-angles to the tops, the edges are, for the most part, not low-index surfaces. (This is symbolized in Fig. 10c by making the edges of layers non-rectangular.) Deposition on the edges of layers, therefore, is deposition on high-index surfaces. There is some reason for thinking that rapid deposition on high-index surfaces does not occur by layer formation, but in a more indiscriminate way. The skeletal shapes of very rapidly growing crystals usually have rounded surfaces; the directions of growth are well defined geometrically, but the actual surfaces are rounded. (Flat surfaces may develop subsequently, and these are low-index surfaces, but during rapid growth the surfaces are rounded.) The absence of flat surfaces during growth suggests that deposition occurs, not by layer formation, but in a more indiscriminate manner. Therefore deposition on the edges of layers may also take place in this way.

There is, of course, a tendency for the edges of layers to become low-index surfaces; this is seen, first of all, as a tendency for the shape of a layer to become more regular as growth slows down; but even when the shape becomes fairly regular, as in the octagonal layers of Fig. 1 (NaCl), it may be doubted whether the edges are surfaces of minimum indices for this reason. If the edges of the above-mentioned octagonal layers were perpendicular to the top (a (100) face, let us say), the surfaces of the edges would be (010) and (011); a (010) face is crystallographically equivalent to a (100) face, and deposition would therefore be equally likely on the edge and the top of the layer; that deposition occurred only or mainly on the edge makes one doubt whether the edge was really (010); it was probably not at right-angles to the top and was therefore a high-index face. Nevertheless, as growth slows down, there is presumably a tendency for the edges of layers to become low-index surfaces. It may be observed that when this happens growth will be very much inhibited, for it will have to wait on the formation of surface nuclei. It is possible that it is in these circumstances the Kossel-Stranski picture of surface nuclei forming on edges and corners is valid; we may well imagine that the most likely place for a nucleus to form is at the edge formed by the top and side of a layer (A in Fig. 10b).

We are thus led to the view that rapid growth of crystals depends on the maintenance of high-index surfaces; if for any reason the surface (so to speak) heals—that is, becomes a low-index surface—growth will be very much inhibited. We shall return to the question of the rate of growth of crystals in Part II.

**Formation of Surface Nuclei.**—Perhaps the most striking and the most important generalization which came out of the many observations made in this work is that, more often than not, the layers were observed to spread, not from the corners or edges of faces, but from the centres of faces. Even when layers were seen spreading from edges or corners, it was sometimes evident that they had “wrapped-over” from contiguous faces. One example may be quoted in which it appeared fairly certain that the point of origin was a corner: on a lead nitrate crystal, a small, rapidly growing face, which showed normal layer growth by spreading from the centre, became smaller and smaller owing to its rapid growth compared with contiguous faces, and was soon eliminated and replaced by a corner; immediately this happened, layers were seen spreading from

this corner over the only contiguous face which could be observed clearly (see Fig. 12). The sequence of events suggests strongly that in these circumstances the layers really did originate at the corner and were not "wrapped-over" from other faces. Nevertheless, the general rule is that layers spread from the centres of faces.

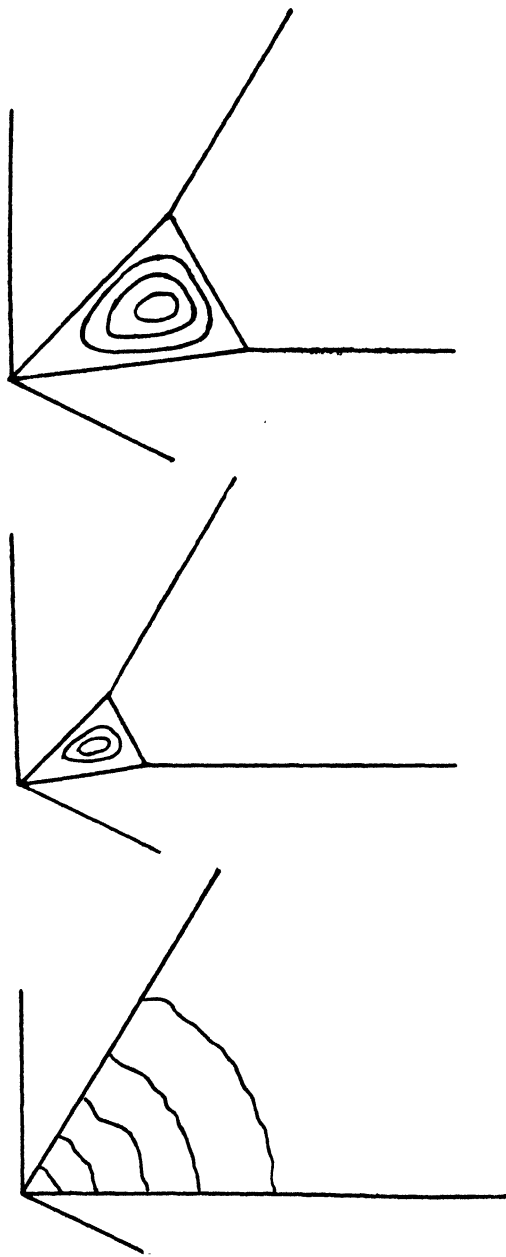


FIG. 12.—Layers spreading from corner of octahedral face of  $\text{Pb}(\text{NO}_3)_2$  crystal, immediately following the disappearance of a small, rapidly growing face.

To the observations made in this work we may add one more, which, although not an observation of growing crystal but a record of the surface structure of a crystal after growth was arrested, seems convincing. Electron microscope photographs taken by R. W. G. Wyckoff (private communication) show, on the surfaces of crystals of a protein (a decomposition product of one of the necrosis viruses), low pyramids of layers. Individual molecules 130 Å in diameter can be seen, and it appears that each layer is one molecule thick; further, the concentric arrangement suggests that during growth layers spread out successively from a point which is not on a crystal edge or corner, just as in the examples seen in the present work. This confirms that, as we have already surmised, the layer-spreading process which we have observed on the scale of hundreds or thousands of ångström gives a correct impression of what happens on the molecular scale.

The spreading of layers from the centres of crystal faces rather than from edges or corners seems surprising, whether one approaches the problem from a consideration of surface forces on the crystal or of the conditions in the solution. If we consider the crystal, the edges and corners are the places where there are most unsatisfied forces, and these are the places where nuclei for the inception of new layers would be expected to form. This idea seems quite generally applicable to all crystals; moreover, in the case of ionic crystals having the NaCl structure, Kossel,<sup>3</sup> Stranski<sup>4</sup> and Brandes and Volmer<sup>5</sup> have calculated the energy-yield in adding an ion to various places on a crystal surface (an atomically perfect plane, the edge of an incomplete layer, etc.) and their calculations indicate that, if we assume the event of greatest energy-yield to be statistically preferred, then the inception of new layers is more likely to occur at edges or corners than in the centre of a face. If we consider the solution surrounding the crystal, it has been shown (see Part II, and Berg<sup>11</sup>) that the supersaturation is greater at the edges of a face than at the centre, and it is at the places where supersaturation is highest that we should expect new surface nuclei to form.

Thus, whether we consider the crystal surface or the solution in contact with the crystal, we are led by current ideas to expect that new layers would start at the edges and corners. But, as we have seen, layers usually do not spread from edges or corners, but from the centres of faces. It is evident that current ideas need revision, or else there is some other factor which overshadows those which have so far been considered.

What are the circumstances at the centre of a face, other than those already considered? If we consider the structure of the crystal surface, a possible cause of the formation of surface nuclei is the existence of cracks or strains; it is noteworthy that when sodium nitrate crystals grow on a calcite cleavage, they grow more freely on cracks than elsewhere; but we know of no evidence indicating (as a general rule) greater imperfection at the centre of a crystal face than elsewhere. If we turn to the solution, a possible clue is given by the study of the concentration distribution round growing crystals of sodium chlorate; according to Berg<sup>11</sup> less solute arrives, per unit area of face, at the edges of a face than in the centre, and therefore, since faces remain nearly flat, surface migration of solute molecules must take place from the centre towards the edges of a face. (In our own earlier work on this aspect, which is considered in Part II, we could not be sure that this was so; but Berg's measurements were perhaps rather more precise.) If it is a fact that the amount of solute per unit area arriving at the centre of a face is greater than at the edges, this may be the reason

<sup>11</sup> Berg, *Proc. Roy. Soc. A*, 1938, **164**, 79. Cp. also Humphreys-Owen, *Proc. Roy. Soc. A* (in press) and This Discussion.

why layers start at the centre ; in spite of the surface migration towards the corners, which tends to relieve the situation, the piling-up of excess solute at the centre is likely to result in additional deposition there—that is, in the formation of surface nuclei. It is suggested in Part II that the tendency for excess solute to arrive at the centre of a face is due to the geometry of the situation : radial inward diffusion to a polygonal crystal necessarily tends to deliver excess solute to the centres of crystal faces.

It is not possible to decide with certainty how far the formation of surface nuclei at the centre of a face is due to surface structure and how far to the disposition of concentration gradients in the solution. The occasional observation of more than one system of layers on a face would appear to favour surface imperfections, but it is not impossible even in these cases that diffusion effects were responsible : convection currents might lead to more than one point of convergence of excess solute on the same face.

**Layer Formation and the Imperfections of Crystals.**—There is a great deal of evidence which indicates that most crystals, even those which are perfectly transparent and have highly perfect faces, are very imperfect in structure. The tensile strengths of actual crystals are only small fractions of what they would be for perfect crystals ; and the intensities of X-ray reflections indicate that the precise structure which exists in small regions is not continued uninterrupted throughout the crystal—there are discontinuities at intervals of the order of 1000 Å. The discontinuities are not at regular intervals ; the idea of a regular secondary structure due to fundamental causes, which was at one time put forward by Zwicky<sup>12</sup> is not now accepted ; there are considerable variations in imperfection of crystals from different sources, as Smekal<sup>13</sup> has shown by measuring mechanical properties of rock-salt crystals, and Lonsdale<sup>14</sup> by divergent-beam X-ray photography of various crystals. It seems likely that these imperfections arise (at any rate partly) from the manner of growth by the spreading of layers across the faces. Successive layers do not necessarily join up perfectly with each other ; it is more likely that cracks will occur, and, moreover, we have occasionally seen on urea crystals layers which, starting apparently in contact with the underlying solid, actually part company with it, leaving a visible crack. Another fact pointing in the same direction is that crystals on which no layers have been seen (that is, on which the layers are too thin to be seen) tend to be more perfectly transparent than those on which thick layers have been seen. These are extreme cases, but the same sort of thing is likely to proceed on a smaller, invisible scale. When layers fail to join up properly, there will be not only a crack between them but also small changes of orientation of the lattice, perhaps best visualized as a slight waviness of each layer. Both types of imperfection are necessary to account for the intensities of X-ray reflections.

Layers, to be visible at all under the conditions of our observations, must be at least several hundred ångström thick ; and those layers whose thickness was measured were 1700–5000 Å thick. For many substances, the layers are on the border-line of visibility, while for many others, they are too thin to be seen. The order of magnitude is about right to account for the fact that many crystals are, as far as X-ray diffraction is concerned, “ideally imperfect.” We have also seen that the thickness of layers is strongly influenced by specific dissolved impurities and the nature of the solvent. There would appear to be scope for investigating the intensities

<sup>12</sup> Zwicky, *Proc. Nat. Acad. Sci.*, 1929, **15**, 253 ; *Physic. Rev.*, 1931, **38**, 1772 ; 1932, **40**, 63.

<sup>13</sup> Smekal, *Physik. Z.*, 1930, **31**, 229.

<sup>14</sup> Lonsdale, *Phil. Trans. Roy. Soc.*, 1947, **246**, 219.

of X-ray reflections of crystals whose growth has previously been observed ; there might be a correlation between layer thickness during growth and the "extinction" effects often found in X-ray reflections ; the layer thickness on urea crystals, for instance, could be varied by growing from mixtures of different proportions of alcohol and water. Again, on many crystals, including NaCl and  $\text{KH}_2\text{PO}_4$ , the layers are thicker towards the edges of a face than at its centre ; there might be a difference between the intensities of reflection of X-rays at these two positions on the crystal face.

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## CRYSTAL GROWTH FROM SOLUTION

### II. Concentration Gradients and the Rates of Growth of Crystals

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When a crystal grows from a supersaturated solution, the concentration of the solution in contact with the crystal is reduced, a concentration gradient is set up, and the crystal is fed by diffusion down this gradient. We may divide the process of crystal growth into two aspects—the "taking" of solute from the solution by the crystal face, and the arrival of more solute by diffusion ; and the rate of growth of a crystal face (thickness deposited in unit time) depends on the factors which control both aspects. A knowledge of the supersaturation at the face is necessary for the consideration of both aspects, for on the one hand it may control the rate at which solute can be "taken" by the face, and on the other, the concentration gradients which are set up depend on the supersaturation at the face as well as on the initial supersaturation of the solution. The diffusive flow of solute is governed by Fick's law, that the rate of diffusion past any point is proportional to the concentration gradient at that point. (Deviations from Fick's law—that is, variations of diffusion constant with concentration<sup>1</sup>—need not concern us at present.)

It was at one time supposed<sup>2</sup> that the concentration at the crystal face sinks to the solubility value ; but in 1903 Miers,<sup>3</sup> by measuring the angle of total internal reflection at a growing crystal face, determined the refractive index of the solution and hence its composition, and established that the solution at the face is very appreciably supersaturated ; he found this was so for three substances—alum, sodium chlorate and sodium nitrate. In later theoretical speculations, such as those of Berthoud,<sup>4</sup> Valetton<sup>5</sup> and Spangenberg,<sup>6</sup> the prevalent idea has been that the rate of growth of a crystal face of a given type is some function of the supersaturation at the face, the underlying conception being that the supersaturation is a measure

<sup>1</sup> McBain and Dawson, *Proc. Roy. Soc. A*, 1935, **148**, 32.

<sup>2</sup> Nernst, *Z. physik. Chem.*, 1904, **47**, 52.

<sup>3</sup> Miers, *Proc. Roy. Soc. A*, 1903, **71**, 439 ; *Phil. Trans. Roy. Soc.*, 1903, **202**, 459.

<sup>4</sup> Berthoud, *J. Chim. Physique*, 1912, **10**, 624.

<sup>5</sup> Valetton, *Z. Krist.*, 1924, **59**, 135, 335.

<sup>6</sup> Spangenberg, *Neues Jahrb. Miner. A*, 1928, **57**, 1197.

of the driving force for deposition. (Crystal faces of different types would be characterized by different rates of growth at the same supersaturation.) The conception seemed reasonable, but there was at that period no experimental evidence to support it, and in 1932 we set out to measure simultaneously the rates of growth of crystal faces, the supersaturation at the faces, and the concentration gradients round the crystal. Miers' method would be difficult to adapt to the exploration of any variation of supersaturation at different points on the same face, and could not give concentration gradients in the solution; a different method was therefore developed. Crystal growth was reduced practically to a two-dimensional process by growing a crystal in a thin film of solution confined between glass plates, and concentration differences were determined from refractive index differences measured by an interference method which was suggested by T. R.

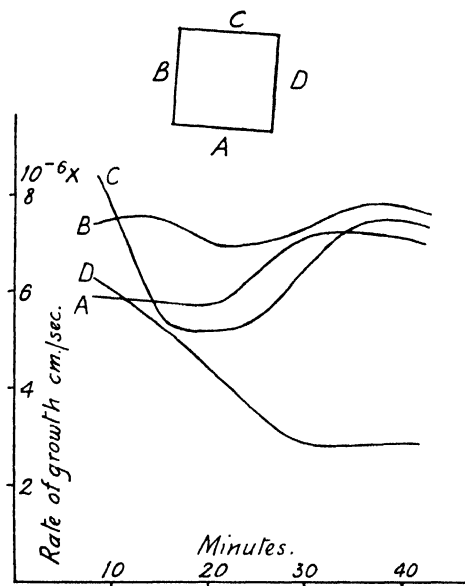


FIG. 1.—Rates of growth of crystal faces, Expt. 1.

Scott, then of this laboratory: the glass plates were half-silvered and not quite parallel, and under the microscope in parallel monochromatic transmitted light, a system of interference fringes was seen, the distortions of which were a measure of changes of refractive index and thus of concentration. Photographs were taken at intervals, and subsequently measured in detail. Sodium chlorate was chosen as a convenient substance for the experiments, as it is cubic, and strongly supersaturated solutions can be obtained in which unwanted additional crystal nuclei are less readily formed than in solutions of many other substances. I shall not give further experimental details, as they are adequately covered in a paper by Berg,<sup>7</sup> who took up this method a little later.

The results of our earliest experiments will not be described in detail, as they are substantially the same as those of Berg. It will suffice to state that the supersaturation was found to vary along any one face, being greatest at the edges and least at the centre; this banished the prospect of discovering

<sup>7</sup> Berg, *Proc. Roy. Soc. A*, 1938, **164**, 79.

any absolute correlation between rate of growth and supersaturation, since faces remained flat in spite of considerable differences of supersaturation at different points. Moreover, the four observed faces of a crystal, though crystallographically equivalent, usually grew at different rates, and no correlation could be found between rate of growth and either the maximum, or the minimum, or the average supersaturation at the face. There was no question of exhaustion of solute at certain faces; in fact, often the most slowly growing faces were in contact with the most strongly supersaturated solution. My purpose now is to reconsider these facts in relation to the phenomenon of layer formation described in the preceding paper, and to describe some experiments in which for various reasons crystal growth was very abnormal; these abnormal experiments are in some ways more instructive than the more normal ones.

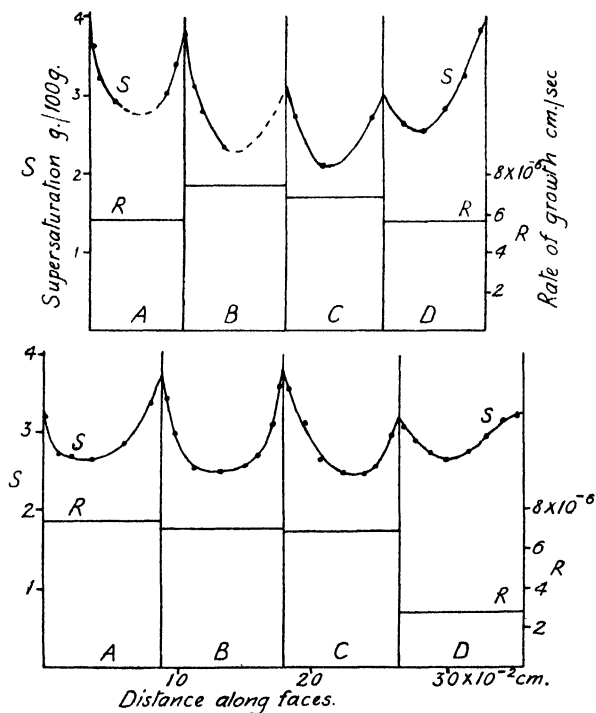


FIG. 2.—Supersaturation along faces, and rates of growth. Expt. 1, at 13 min. and 36 min. Initial supersaturation of solution, 7.77 g./100 g. solution.

### Experimental

Three experiments will be described, which were the most remarkable and instructive of those carried out; they were all done in 1932. In the first, the principal point of interest is in the fact that the rates of growth of the four observed faces (see Fig. 1) did not slowly and steadily diminish with time as in some other experiments (see Expt. 2 below); the rate of growth of one face (D) diminished rapidly, within about 20 min., to half its initial value, while those of the other three faces first decreased and then increased again. The increase was most marked for faces A and B which were adjacent to D. Face A, which was at first the slowest, became the most rapidly growing. This was certainly not due to exhaustion of the solution near face D, for in this experiment the initial solution was exceptionally strongly supersaturated (7.77 g.  $\text{NaClO}_3$  per 100 g. solution), and the concentration at the crystal faces was everywhere far above





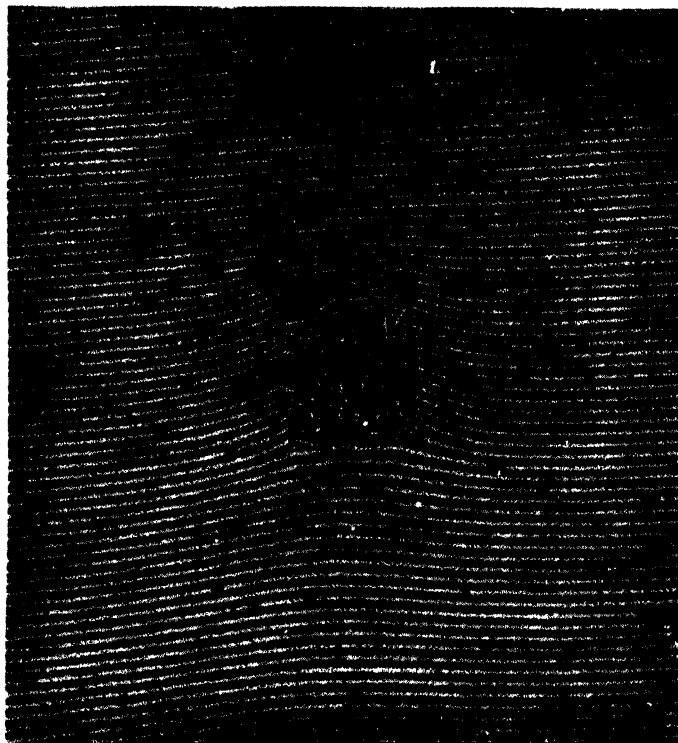


FIG. 3.—Crystal of sodium chlorate (the cracked crystal of Expt. 2) growing in thin layer of solution between nearly parallel half-silvered mirrors, illuminated by parallel monochromatic light.

saturation for the whole duration of the experiment. The variation of supersaturation along the faces, shown in Fig. 2, was of the same type as in our earlier experiments and those of Berg, and there was again no correlation between rate of growth of a face and the maximum or minimum or average supersaturation. The changes in rate of growth of the faces give the impression that the total amount of solute reaching the crystal was limited but was redistributed during the course of the experiment.

Faces A, B and C in this experiment were not entirely flat; face B was very slightly convex throughout, while A and C at different times exhibited temporary steps, when one half of a face gained on the other; but these steps were soon eliminated (they are ignored in Fig. 1, which gives the average rate of growth for each face). This demonstrates that in normal circumstances there is an influence (presumably the layer-spreading process) which tends to keep a crystal face nearly flat during growth, and is even able to overcome an appreciable temporary departure from flatness.

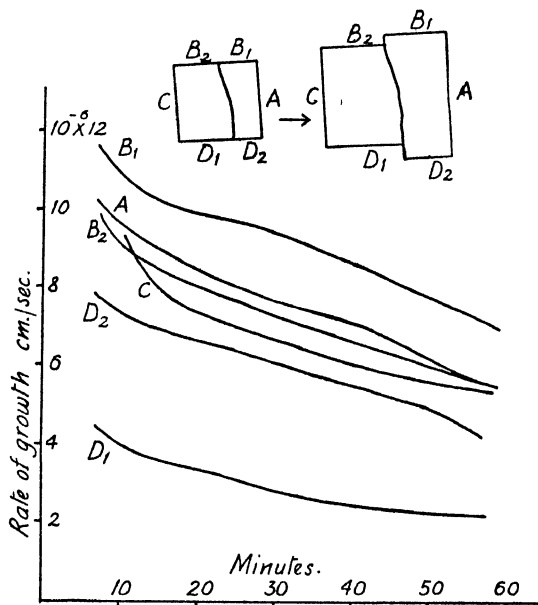


FIG. 4.—Rates of growth of faces of cracked crystal, Expt. 2.

The course of events in Expt. 2 is very relevant to this same point. In starting the experiment (probably when the upper mirror was put in place) the crystal became cracked right across; each of the two opposite faces which were cracked behaved as two independent faces, the two halves growing at considerably different rates. One photograph is reproduced in Fig. 3 to illustrate the sort of experimental material on which these results are based. Fig. 4 shows the rates of growth, which in this experiment show a steady decline with time, and Fig. 5 shows the supersaturation along all the faces at two stages (there was very little change in this respect in the duration (67 min.) of the experiment).

The independent growth of the two halves of a cracked face underlines what was said in discussing Expt. 1; presumably the layers were not able to bridge the crack, and each half of the face was therefore free to respond independently to whatever changes occurred either on its surface or in the solution in contact with it.

In Expt. 3 the course of events was still more abnormal. As in all the experiments, some initial dissolution of the seed crystal occurred when warm solution came in contact with it; but in this case one corner was dissolved more than the others, and by the time the assemblage reached laboratory temperature,

the crystal had a small (110) face on one corner in addition to the usual four 100 (cube) faces. This (110) face grew faster than any of the cube faces, and soon eliminated itself, its rate of growth increasing towards the end. The rates of growth of the cube faces showed the remarkable changes recorded in Fig. 6; face A soon stopped growing altogether, while at the same time the rate of growth of B very much increased. Face A did not grow at all for a whole hour afterwards; the changes in the rates of growth of the others were somewhat similar to those which occurred in Expt. 1. The supersaturation along the faces at two points of time in the crucial period (at 8 min. and 13 min.) is shown in Fig. 7 and 9, and the concentration distribution round the crystal at the same times in Fig. 8 and 10.

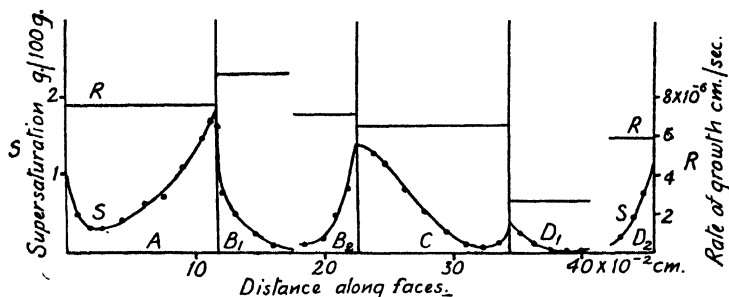


FIG. 5.—Supersaturation along faces, and rates of growth. Expt. 2, at 32 min. Initial supersaturation of solution, 4.9 g./100 g. solution.

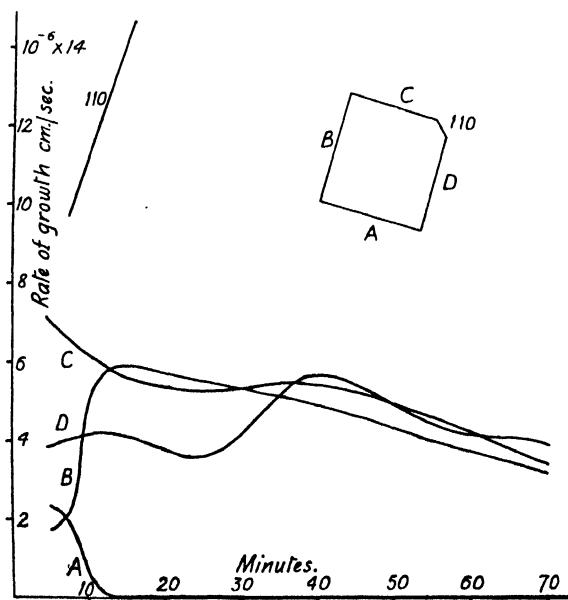


FIG. 6.—Rates of growth of faces, Expt. 3.

The course of events in this experiment forms the most striking demonstration of the lack of correlation between the rate of growth and supersaturation, for face A was in contact with strongly supersaturated solution, and yet stopped growing; in fact, by the time it had stopped growing, the average concentration of solution in contact with it was greater than for any of the other three faces (Fig. 9).

The diagrams of distribution of concentration (Fig. 8 and 10) suggest that,

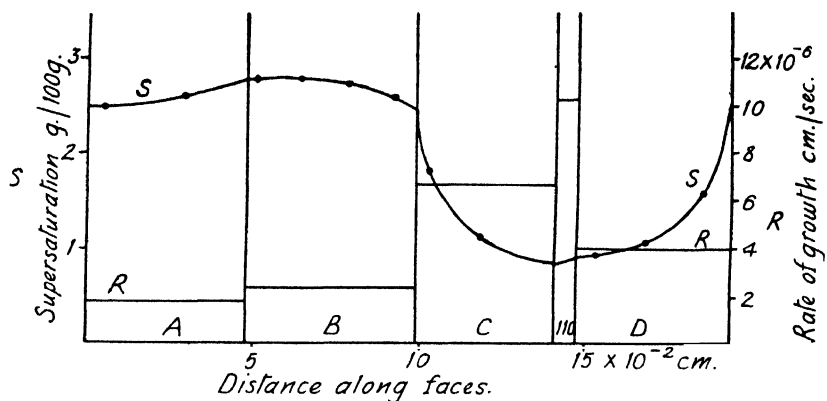


FIG. 7.—Supersaturation along faces, and rates of growth. Expt. 3, at 8 min. Initial supersaturation of solution, 3.36 g./100 g.

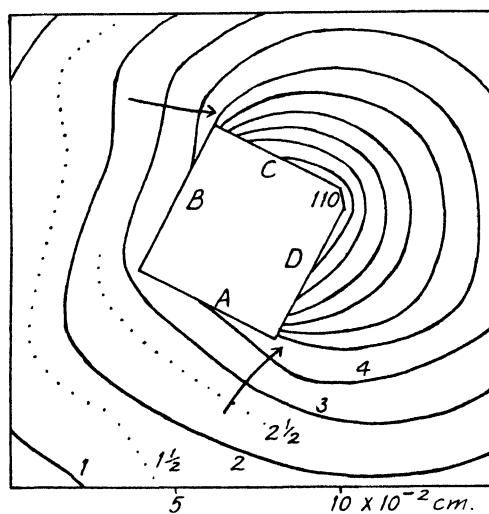


FIG. 8.—Equal-concentration contours. Expt. 3, at 8 min. Contours drawn at intervals of 0.246 g./100 g.

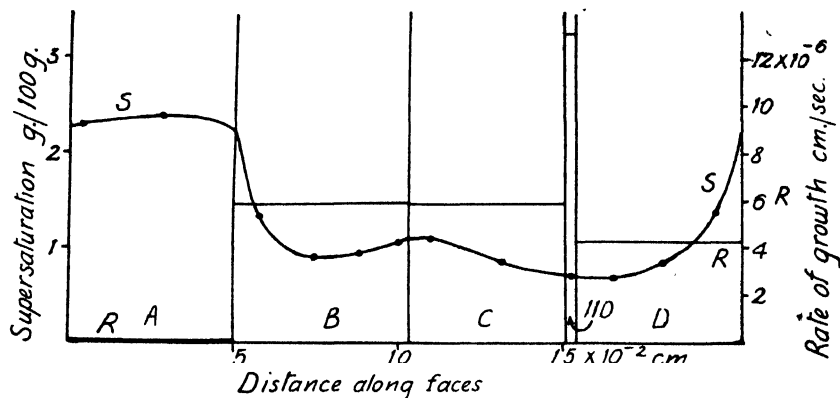


FIG. 9.—Supersaturation along faces, and rates of growth. Expt. 3, at 13 min.  
E\*

at first, the avidity of the rapidly growing (110) face caused an extensive reorganization of the concentration distribution round the crystal; near the (110) face, not only were the gradients steepened, but also the supersaturation near the crystal was much reduced, and the effect was to shift the diffusion centre (the region towards which solute particles were diffusing) away from the centre of the crystal and towards the (110) corner face; it may be that this is the reason why, at this period, faces C and D which were adjacent to the (110) face were growing faster than A and B—solute was diverted from A and B towards C and D, as indicated by the arrows in Fig. 8. If this were all, we should expect faces A and B to continue to grow at about the same speed; but in fact A stopped growing altogether while B's rate of growth increased until it was about the same as that of C. These changes are similar to, but more extreme than, those which occurred in Expt. 1; and they raise in the most acute manner the central question: what is it that determines the speed at which a cube face of sodium chlorate grows? It is evident that the supersaturation at the face is not the determining factor. Are the extreme variations due to some other condition in the solution, or to changes on the surface of the crystal?

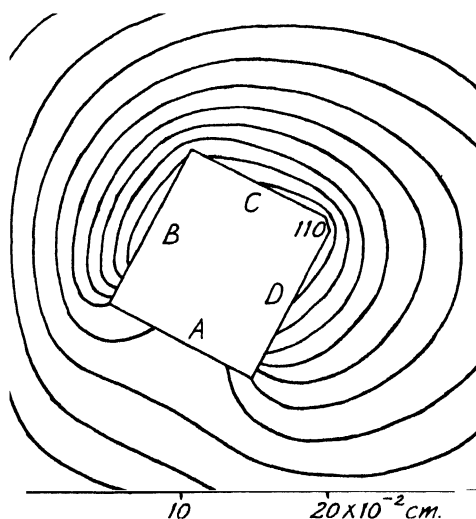


FIG. 10.—Equal-concentration contours. Expt. 3, at 13 min. Contours drawn at intervals of 0.246 g./100 g.

Dr. S. P. Humphreys-Owen,\* who has recently been studying the growth of sodium chlorate crystals by the same method, has observed phenomena similar to those described here—extreme variations of the rates of growth of the four observed faces, including the complete stoppage of growth of one or more faces. He has also observed the restarting of growth of a stopped face. All these phenomena must be considered in any attempt to frame a theory of crystal growth from solution.

Berg,<sup>7</sup> in considering the fact that crystallographically equivalent faces grew, in his experiments, at different rates, was inclined to attribute the differences to the presence of local traces of impurities. This suggestion does not seem acceptable. Dissolved impurities would affect all faces equally. Undissolved impurities might become localized; but it is difficult to see how they could cause the effects observed. Impurities may or may not be built into the crystal; if they are built in, the poisoning effect would be temporary, because fresh material, deposited on the impurity, would create a fresh surface free from impurity; if they are not built in, they remain near the surface, rejected by each layer but able to hinder growth by their continued presence; but it is difficult to

\* Humphreys-Owen, *Proc. Roy. Soc. A* (in press).

believe that the effects would be confined to one particular face for any length of time—neighbouring faces would be affected. Moreover, how are we to explain the fact that the rates of growth of A, B and C in Expt. 1 increased while that of D decreased? It is unlikely that impurity would be built in and covered over on three faces but not on the fourth. It is equally unlikely that impurity would migrate towards D and away from the three other faces.

If we reject explanations based on local surface contamination, we must consider whether the phenomena can be explained by any other solution condition, or by surface changes arising from within the crystal (such as strains or cracks). On solution conditions, we may observe that the only condition other than supersaturation at the face (which is clearly not the controlling factor) is the concentration gradient at the face. Concentration gradients arise in the first place because the crystal, so to speak, "takes" solute out of the solution and makes its own gradient; but suppose the gradient is disturbed by convection currents: will this affect the rate of growth of the crystal face, irrespective of the supersaturation at the face? Suppose the concentration at the face rises and the gradient is partly levelled: will the rate of growth decrease? It need not: the crystal face might continue to "take" solute from the solution at the former rate, and this would restore the gradient to its former value. But the sequence of events in Expt. 3 suggests that a local steepening of gradient due to the rapid growth of a small (110) face led to an increased rate of growth of the neighbouring cube faces; and this suggests that changes of gradient due to external causes such as convection currents might similarly affect the rates of growth of cube faces. The existence of slow currents (due to variation of density of the solution and to heat of crystallization) was confirmed in one experiment by watching the movement of colloidal particles deliberately put into the solution; such movements might take unsymmetrical paths and thus affect different cube faces unequally.

We are thus led to enquire whether there is any justification for regarding diffusive flow of solute as not merely an effect following on surface reactions controlled by other factors, but on the contrary as a process which (once it is started) has a positive influence of its own. In discussing this question, possible relations between the phenomena of layer formation and the conditions in the solution will be considered.

### Discussion

**Surface Profile in relation to Supersaturation at the Face.** The fact that the supersaturation of the solution at a crystal face varies all along the face shows that the possibility discussed by earlier theorists that the rate of growth ( $G$ , the thickness\* of solute deposited on a face in unit time) is some function of the supersaturation  $S$  at the face is not correct in its simplest form, since a crystal face usually remains substantially flat in spite of considerable differences of supersaturation at different points. But this theoretical possibility was based on the assumption that a crystal face is a uniform surface. It has, however, been pointed out in Part I that a crystal face may be effectively a high-index surface at the centre and predominantly a low-index surface near the edges; consequently the surface forces vary from the centre to the edge of a face, and if one postulates a relation

$$G = Kf(S),$$

it must be with the proviso that  $K$  may vary all along any one face; it would be high at the centre of a face where there is a high-index surface, and low towards the edges where there is predominantly a low-index surface. It may be that supersaturation and surface character are mutually adjusted so that for any one face at a particular time  $Kf(S)$  is constant. Furthermore, the lack of correlation between the rates of growth of different faces and

\*  $G$  represents the average thickness deposited all over the face, ignoring the fine structure of the surface.

the supersaturation might be due to differences of surface profile which completely mask the influence of supersaturation. The experimental evidence either means this, or else it means that the magnitude of the supersaturation at the face plays no part in determining the rate of growth (apart from the basic fact that no growth occurs unless the concentration at the face exceeds the saturation value).

There appears to be little hope of securing experimental evidence on this question, for it is difficult to see how to discover the crystallographic character of layer edges while growing. But even if it were demonstrated that there is a definite relation between the character of the surface, the supersaturation and the rate of deposition, it would still leave the main problem unsolved : at a given supersaturation the rate of growth might have a wide range of values depending on the surface profile which is set up during growth. It is necessary to enquire what determines the type of surface which is set up. It is likely that the key to the situation is to be found at the centre of a crystal face, where layers normally originate.

**Layer Formation in relation to the Diffusion Field.** It is a striking fact that normally layers spread from the centres of crystal faces, where the supersaturation is lowest. (It is assumed here that the findings on sodium chlorate are typical. A few preliminary experiments on one other substance, potassium ferricyanide, showed that for this crystal also the supersaturation is lowest at the centres of the faces. Further work on other substances is desirable.) The magnitude of the supersaturation evidently does not control the inception of layers ; and this is consistent with the facts about the rate of growth of faces. The other solution conditions which may vary over a crystal face are the concentration gradients which control the diffusive flow of solute ; and we therefore turn to a consideration of the diffusion field.

According to Berg, more solute arrives at the centre of a face than at the edges, and since the face remains flat, the excess must be dissipated by surface migration. While it may be doubted whether the excess is as large as the 25-50 % stated by Berg (the accuracy of measurement of gradients is not great enough to give confidence in the magnitude, which would mean an enormous surface migration), nevertheless, if we may accept the indication of an excess at the centre rather than at the edges, this suggests an obvious explanation of the inception of layers at the centres of faces. Moreover, the arrival of excess solute at the face centre can hardly be attributed to events on the crystal surface but must be due to the diffusion process. This line of thought leads us to ask whether radial inward diffusion to a polyhedral crystal necessarily tends to deliver excess of solute to the centres of faces.

If there were no surface migration, the crystal surface would "take" from the solution uniform amounts of solute all along the face, and this would impose on the diffusion field a particular concentration distribution capable of supplying uniform amounts of solute to the surface. But if surface migration may occur, even if only to a small extent, the diffusion process is not tied down to delivering uniform amounts of solute, since any non-uniformity can be dissipated by surface migration. In these conditions, radial inward diffusion to a polygonal crystal plate is unlikely to deliver uniform amounts of solute along each face : the amount arriving at the corner is likely to be different from that arriving at the centre. Precise mathematical treatment of this problem does not appear possible, but by the following approximate numerical procedure (suggested to the writer by Sir Cyril Hinshelwood) it is possible to draw some instructive conclusions.

Round a square representing the crystal plate, the field is divided into small squares, each containing a number representing supersaturation. At first the numbers are all equal ; to represent the start of crystal growth,

the numbers in the squares next to the crystal are all reduced by the same amount, representing the amount of solute taken out of the solution by the crystal in unit time. The diffusion process is represented by calculating the change in each figure in unit time due to transfer of solute to or from each of the neighbouring squares, using an arbitrary diffusion constant. Alternate stages of deposition and diffusion are then carried out; in doing this, various assumptions about deposition can be made—for instance, uniform deposition along the face at a rate which can be either constant or diminishing with time (these assumptions corresponding to absence of surface migration), or deposition from each square at the rate at which solute arrives by diffusion (this corresponding to the assumption of surface migration which dissipates any non-uniformity of arrival). The diffusion field spreads outwards with each successive stage, just as it does in practice. When uniform deposition was enforced at a rate slowly declining with time (to imitate the experimental conditions), this led to the establishment of a diffusion field similar to that found experimentally (in normal undisturbed circumstances), with equal-concentration contours which in the outer region are nearly circular, but near

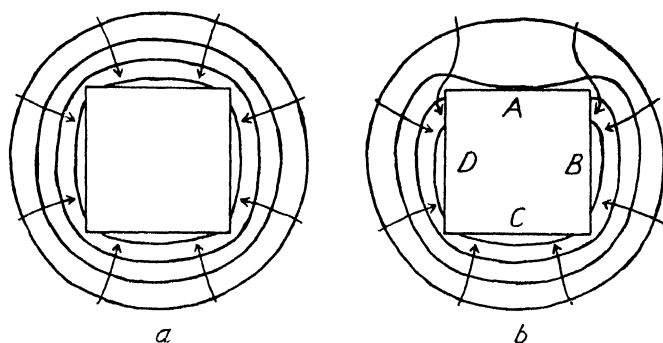


FIG. 11 *a*.—Form of equal-concentration contours in symmetrical growth, obtained by calculation. *b*. If, at face A, a decline of gradient and rise of concentration occurs, this leads to a greater convergence of solute on B and D.

the crystal have a shape which is a compromise between square and circular (as in Fig. 11 *a*). The amount of solute arriving at each square along the face was nearly uniform, but there was a persistent small deficiency at the corner: thus, at each diffusion stage, slightly too little solute arrived near the corner, but at each deposition stage, uniform amounts were taken out of each square. The procedure was then altered, to study the tendency of the diffusion process when not tied down to uniform delivery at the face; whatever solute arrived at each point was assumed deposited there. This had the effect of making the deficiency of arrival at the corner more and more marked at each successive stage, and the inner concentration contours became gradually more nearly circular.

This procedure is slightly unrealistic, in that the "crystal" does not actually grow; and the operations are approximate; but it does represent the essentials of the present discussion, and the effect appears to be a genuine indication of the tendency of the radial diffusion process to deliver less solute to the corners than to the centres of crystal faces. We may regard this as due to the tendency of the diffusion field towards the circular symmetry appropriate to radial inward diffusion: the outer contours which are nearly circular try to impose their symmetry on the inner ones; and the more nearly circular are the inner contours, the greater the excess of solute arriving at the centre of a face. The tendency would be still more marked



in three-dimensional diffusion, and the excess arrival at the centres would build up as far as surface migration allows.

This conclusion not only suggests the explanation of the inception of layers at face centres which has already been stated, but it also has implications on the question of changes in rates of growth; for if the normal inception of layers at face centres is due to diffusive convergence of excess solute there when the diffusion field round the crystal is undisturbed, then any disturbance of the field by convection currents is likely to change the rate of inception of layers unequally on different faces. The suggestion already made, that externally caused gradient changes may be responsible for changes in rate of growth (irrespective of supersaturation at the face), is thus supported.

The simplest way of regarding the inception of layers at face centres (if the present views are correct) is the following. If the shape of the crystal depended on the diffusion process alone, it would be spherical; but in fact the crystal opposes this tendency and forms nearly flat faces. The formation of a low pyramid of growing layers represents the attempt by the diffusion process to make the crystal spherical—an attempt which is not very successful, since the layer system on a growing crystal face is a scarcely perceptible departure from flatness.

The example quoted in Part I, in which layers spread from the corner of a lead nitrate crystal following the disappearance of a small rapidly growing (presumably high-index) face there, is consistent with these views. A rapidly growing face sets up a steep, strongly convergent diffusion field (cp. the 110 face in Expt. 3 above); as soon as this face disappears and is replaced by a corner, this field initiates layers on the normal faces which meet at that corner.

**Surface Profile in relation to Concentration Gradients.** The problem of rate of deposition on a crystal surface will now be approached in a different way, in an attempt to understand more closely the relation between the character of the deposition surfaces and the solution conditions. It has been noted in Part I that deposition takes place on the edges of layers which are apparently high-index surfaces; at the centre of a crystal face the layers are sometimes so thin that this part of the surface may be regarded as a low pyramid of vicinal faces, while towards the edges of the face where the layer edges are thicker, the surfaces are often irregular—possibly irregular on the molecular scale; in any case the term “high-index surface” covers all deposition surfaces. Further, there is a tendency for such surfaces to “heal”—that is, for deposition to occur in such a way that the new surface has lower indices; the very formation of thick layers is the first symptom of this tendency, and its further progress is indicated by the tendency for the layer periphery to become more regular as growth slows down. The slow decline of rate of growth with time in undisturbed conditions (see Fig. 4) may be due to gradual healing. Rapid crystal growth, in fact, appears to depend on the maintenance of sensitive high-index surfaces; if the surfaces were to heal completely, growth would be severely inhibited; it is possible that the complete stoppage of growth of faces which has sometimes been observed is due to complete healing.

The outstanding question which arises is the following: what is it that prevents complete healing in normal circumstances? If a crystal having imperfect surfaces is put into a supersaturated solution, why is it that the first solute molecules do not deposit in such a way as to make low-index surfaces which would then grow no further? The supersaturation is certainly not the controlling factor here, because stopped faces are usually found to be in contact with the strongest solution; and it is difficult to imagine any

other solution condition except the diffusive flow of molecules set up by the initially formed gradient. It may be that if solute molecules arrive fast enough, they are deposited in an indiscriminate way so that high-index surfaces are maintained.

This suggestion appears to imply that when solute molecules are moving towards the crystal surface, the increased component of Brownian motion towards the surface increases the chance of deposition on sites which preserve a high-index surface. The change in the component of molecular velocity towards the surface which diffusive flow implies is, of course, small; but the chance of deposition on such sites might increase appreciably with quite a small change in the component of motion towards the surface. The chance of deposition on a site of particular crystallographic character may depend on the proportion of molecules having velocity components towards the surface which exceed a critical value; and the critical value may be quite delicately related to the crystallographic character of the site.

If it is true that healing is prevented by a sufficiently rapid diffusive flow of molecules towards the surface, we may imagine that exaggerated effects may follow a local levelling of the gradient by convection currents: the surface partially heals, and becomes less capable of receiving solute, and this may lead to a further decline of gradient. How far such a progressive change would go cannot be predicted, but the total stoppage of growth, which sometimes occurs, indicates that it may go to extreme lengths in this direction. Further, if the rate of growth of one face decreases, the levelling of gradients there will lead to a greater convergence of diffusing solute on neighbouring faces (Fig. 11*b*), and consequently to an increase in their rate of growth; the differences between the rates of growth of neighbouring faces of the same crystal are thus still further exaggerated. (This is presumably part of the explanation of the course of events in Expt. 1 and 3.) The restarting of growth of a stopped face which has been observed by Humphreys-Owen might be explained in a similar way as being due to the external building-up of a gradient and consequent diffusive flow which leads to the formation of a layer nucleus; or alternatively this might be truly a chance phenomenon: on a perfect cube surface in contact with homogeneous supersaturated solution, the chance of formation of a layer nucleus is very small but not zero.

**Conclusion.** Much of the foregoing discussion has been concerned with the diffusion field and the surprisingly important part it appears to play in layer formation: the role which supersaturation might have been expected to play is in fact (according to the present interpretation) taken over by the concentration gradients. Nevertheless, the part played by diffusive flow is only one side of the picture; indeed, chronologically it is a secondary part. When a crystal is put into a homogeneous supersaturated solution, there are at first no gradients; it is only when the crystal "takes" solute out of the solution that gradients are created; the "taking" of solute out of the solution by the crystal is the primary process. It is perhaps here that the magnitude of the supersaturation at the surface plays its part: the initial rate of deposition may depend on the supersaturation as well as on the nature of the surface. (The growth of a crystal nucleus spontaneously formed in a solution is a different matter; the "taking" of solute from the solution is part of the process of nucleus formation, which will not be considered here.)

The extreme importance of the nature of the surface has already been emphasized; it is possible that if a sodium chlorate crystal with perfect cube faces were put into a supersaturated solution, it would not grow at all, or at any rate the beginning of growth might be delayed an indefinite time.

(It would be difficult to test this, because any seed crystal grown for the purpose, when taken out of its solution, is unlikely to have perfect surfaces even if it had them originally, owing to drying of mother liquor which would be likely to give irregular surfaces.)

The present work gives no information on the possible connection of rate of deposition on a given surface with the magnitude of the supersaturation, because the precise crystallographic character of the deposition surfaces is not known. It does not seem possible to determine the character of the deposition surfaces in normal growth; but it might be worth while to study growth on more extensive high-index surfaces which are deliberately created by partial dissolution. The character of the surfaces would change from the moment growth started, and it would therefore be necessary to measure the rate of growth from the earliest possible moment and extrapolate back to zero time. Only by observing growth in the earliest possible stage is it likely that any significant relation between character of surface, supersaturation and rate of growth would emerge.

The conception of crystal growth developed in this paper is not unlike the current conception of certain chain polymerization reactions depending on activated molecules, where the initiation of chain formation depends on activation (photochemically or by free radicals), and its continuation depends on the maintenance of activated chain-ends. In crystal growth, high-index surfaces appear to be the active surfaces which are capable of adding on further molecules; the beginning of growth depends on the presence of such surfaces, and its continuation depends on their maintenance. The problems of rate of crystal growth can be divided into a study of the factors controlling initiation (the magnitude of the supersaturation at the surface may play a part here), and those controlling the maintenance of active surfaces (in which the rate of diffusive flow of solute appears to play an important part). Deposition on high-index surfaces appears to be the key to problems of crystal growth.

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## THE GROWTH OF INDIVIDUAL FACES OF CUBIC SODIUM CHLORATE CRYSTALS FROM AQUEOUS SOLUTION

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Bunn, in hitherto unpublished work, and Berg<sup>1</sup> grew crystals of NaClO<sub>3</sub> alone in a thin film of aqueous solution between optically-plane glass plates. Growth was constrained to the four faces in the plane of the film. The rates of advance of these faces were measured together with the concentration

<sup>1</sup> Berg, *Proc. Roy. Soc. A*, 1938, **164**, 79.

contiguous to them, the latter being deduced by means of interference fringes formed in the solution surrounding the crystal. The results obtained were unexpectedly complex. It was found that :

- (i) the concentration was not uniform along a face, but was lowest at its centre, rising on each side to the corners ;
- (ii) the component of the concentration gradient normal to the face (hereafter called the normal gradient), which is proportional to the exit of solute per unit area from the solution, was also not uniform. It was highest at the centre and fell towards the corners ;
- (iii) faces of one crystal, though all of the same type and initially in contact with solution of the same strength, usually grew at different rates and sometimes stopped growing altogether ;
- (iv) there was a tendency for the slower growing faces of one crystal to be in contact with solution of higher concentration.

Berg suggested that (ii) implied the existence of a transport of solute laterally along the face in a layer too thin to be observed. For without such a transport the growth of the face as a plane would be inexplicable.

The present author<sup>2</sup> has obtained some new results with  $\text{NaClO}_3$ , using the same technique, which are reviewed and discussed in this paper.

### Predictions from Diffusion Theory

With a given supersaturation far from the crystal as one boundary condition, and some selected boundary condition at the crystal surface, the diffusion of solute to the crystal is determined and its rate of growth can be approximately calculated and compared with experiment. In order to render the treatment mathematically practicable two approximations were made ; the polygonal perimeter of the crystal was replaced by a circular perimeter (under the experimental conditions the diffusion field is two-dimensional). Secondly, the difficulty caused by the non-existence of a stationary state was overcome by neglecting the first moments of rapid growth and treating only subsequent periods, in which the increase in size of the crystal and of the surrounding zone of non-uniform concentration was regarded as slow.

- Let  $\rho$  = density of the solid crystal,
- $a$  = radius of the crystal,
- $t$  = time,
- $c_a$  = concentration at the crystal, assumed uniform,
- $c_s$  = solubility.

The diffusion constant,  $D$ , of the solute was defined from Fick's law : mass of solute transported per second across unit area equals the product of  $D$  and the concentration gradient normal to that area.

Since it appeared from Bunn's results that differently growing faces of one crystal were associated with different values of the contiguous concentration, Berthoud's equation<sup>3</sup> was employed as the boundary condition at the crystal surface, namely

$$G = k(c_a - c_s), \quad \dots \quad (1)$$

where  $G$  is the mass per second per unit area incorporated into the crystal, and  $k$  is a constant of the surface which can be different for different faces.  $c_a$  takes a value dependent simultaneously on  $k$  and on the external diffusive environment.

For the far boundary condition it was assumed that a constant, uniform

<sup>2</sup> Humphreys-Owen, *Proc. Roy. Soc. A*, 1949, **197**, 218.

<sup>3</sup> Berthoud, *J. Chim. Physique*, 1912, **10**, 625.

concentration,  $c_\infty$ , existed at and beyond a radius  $r_0$  measured from the centre of the crystal. It was found experimentally that, except for the first moments of growth,  $r_0$  increased slowly at a rate such that its ratio to the diagonal of the crystal remained constant. The ratio  $r_0/a$  was therefore taken as constant in the calculation, and the disturbance of the stationary state caused by the time-dependence of  $r_0$  and  $a$  was ignored. With this assumption and the above boundary conditions it can be shown<sup>2</sup> that:

$$\rho \cdot da/dt = k(c_a - c_s), \quad . \quad . \quad . \quad (2)$$

$$a \cdot da/dt = \frac{(D/\rho)(c_\infty - c_a)}{\ln(r_0/a)}, \quad . \quad . \quad . \quad (3)$$

$$c_a = \frac{(1/a)(D/k)c_\infty + c_s \ln(r_0/a)}{\ln(r_0/a) + (1/a)(D/k)}. \quad . \quad . \quad . \quad (4)$$

The radial rate of advance declines with time as  $a$  increases, and depends on the ratio  $D/k$ . If  $k$  is large compared with  $D$ ,

$$(4) \text{ becomes } c_a = c_s, \\ \text{and } (3) \text{ becomes } ada/dt \propto (c_\infty - c_s).$$

This is the historical assumption of Nernst for growth from solution, in which the concentration at the crystal surface is brought down effectively to the saturation value and the facial rate of advance depends only on the diffusion geometry. In this case, (2), which represents the dependence of the rate of advance on the crystallographic factor  $k$ , then becomes indeterminate with  $(c_a - c_s)$  tending to zero as  $k$  tends to infinity.

For comparison with experiment a face is regarded as the arc of a circle, and the non-uniformity of concentration along it observed by Bunn is neglected. Of course, it is implicit that central symmetry be preserved; when faces of one crystal grow at different rates and are in contact with different concentrations there will be a redistribution of lines of flow round the crystal and the above equations will lose some of their validity.

### Experimental and Results

Bunn's observation of different facial rates of growth in one crystal was confirmed, but it was found that certain faces did sometimes grow in accordance with the Nernst assumption. In these cases the distance  $y$  advanced by a face (made equivalent to  $a$  by a suitable numerical factor) obeyed (3) quantitatively for the case  $c_a = c_s$ , where  $c_a$  was taken as the concentration at the face centre. This indicated that the centre concentration, to be called  $c_m$  hereafter, is the important one, and that Bunn's distribution of concentration along the face is a "fine structure" effect of the growth mechanism and can be replaced by  $c_m$  when calculating rate of advance.

Often, however, faces were found which grew at rates less than calculated under the Nernst assumption. In these cases the value of  $c_m$  was not that corresponding to saturation but higher. This is qualitatively in accordance with (3); for, with a given  $c_\infty$ , it predicts an inverse correlation between  $ydy/dt$  and  $c_m$ . The quantitative agreement was not good, because crystals with all four faces with the same rate of advance and concentration distribution were never encountered, and the distortion of the concentration field caused by differently behaving faces modified the concentration at each face. The other prediction, the time-dependence of  $c_m$  by reason of the term  $1/a$  in (4), would be falsified both by lack of central symmetry and by the only partially justified assumption of a stationary state, and was not observed.

But these results, as far as they go, are of interest in their demonstration that the Nernst mode of growth is observed only with certain faces of a crystal bounded by faces all of the same type. Other faces appear to have a smaller value of the Berthoud constant  $k$ , and behave as if they were "hindered" in some way. It is still possible that they obey the condition (1), but to test this rigorously it will be necessary to isolate one face from the others of the same

crystal, and also to confine the inflow to one dimension so that the true diffusion equation in  $\partial c/\partial t$  can be solved.

Nevertheless, it will be convenient in the discussion to assume the truth of (1) and to say that the hindered faces have a smaller value of  $k$  than others. Faces did not always preserve the same  $k$  during an experiment. Sometimes it changed, and when this happened the change was observed to be sudden and discontinuous. The concentration at the face would suddenly rearrange itself to a new distribution. This points to a definite, abrupt event at the crystal surface and will have important bearing on any physical hypothesis put forward to interpret "hindrance."

The variation of concentration in the vicinity of a face was studied quantitatively, and both the concentration and its normal gradient  $g$  were expressed empirically in terms of  $x$ , the distance along the face from its centre,  $R$  the rate of advance of the face, and other observables. Neglecting distortion caused by adjacent faces having different behaviour, it was found that  $g$  could be expressed by the parabola:

$$g = R\{(\rho/D) + [q - (\rho/D)] [1 - 3(x^2/l^2)]\}, \quad (5)$$

where  $l$  is the half-length and  $q$  is an experimentally observed constant. Units and typical values were:

$$\begin{aligned} R &= 10^{-5} \text{ cm./sec. (typical),} \\ l &= 0.015 \text{ cm. (typical),} \\ D &= 1.7 \times 10^{-7} \text{ g./cm. sec.,} \\ q &= 1.62 \times 10^7 \text{ sec./cm}^2, \\ \rho &= 2.5 \text{ g./cm}^3. \end{aligned}$$

$g$  was expressed in change of concentration per cm., where concentration is g. solute per 100 g. solution.

If there is a lateral flow of solute along the face, as suggested by Berg, its value at any point can be derived from (5) as long as (a proviso pointed out by F. C. Frank) the diffusion of solvent down the gradient  $\partial c/\partial x$  is neglected. With the above figures the flow is such that at the face centre about 9 % of the intake from outside does not crystallize locally. For an estimate of the physical significance of this lateral flow the magnitude of the transport across unit area must be known. This requires knowledge of the thickness of the layer in which the flow takes place. The layer is not thick enough to cause any discontinuity in the interference fringes terminating at the crystal, but nevertheless the upper limit to the thickness is uncertain within wide limits because refraction effects at the crystal edge prevented observation much closer than  $10^{-4}$  cm. from the face. Berg (*loc. cit.*) suggested that the layer might be a Volmer adsorbed phase. If the thickness is taken to be, say,  $10^{-7}$  cm., on the assumption of a Volmer layer one molecule thick, the transport of solute across unit area works out at about 6000 times the diffusive transport in the outside solution. This indicates a very high coefficient of diffusion in the layer.

Of course, a lower coefficient of diffusion is derived if a greater layer thickness is assumed, but it is difficult to find grounds for postulating a mobile layer many molecules thick.

The absolute gradient, normal to lines of equal concentration, was found to be uniform along a face, and this fact can be used in conjunction with (5) to derive an expression for the concentration,  $c$ , as a function of  $x$ . This is:

$$c = c_m + lR \sqrt{\frac{\alpha^2}{\beta}} \left\{ 1 - \left( 1 - \frac{\beta}{\alpha} \cdot \frac{x^2}{l^2} \right)^{1/2} \right\}, \quad (6)$$

where  $\alpha = 2q/3$ , and  $\beta = q - \rho/D$ .

Eqn. (6) represents a very large effect, as can be seen from the fact that the concentration difference between the face centre and the corners is about  $1/4$  of the supersaturation of the solution far from the crystal.

Some  $x$ -dependent property of the face is required to explain (6), and if, as suggested by Frank, an intermediate layer between the face and the solution is not acceptable as a source of such a property, something must be put in its place. Bunn has favoured the existence of vicinal planes on a growing face as an explanation, but it is doubtful whether such a hypothesis can be found capable of dealing quantitatively with (6).\*

The non-uniformity of concentration is observed whether or not hindrance is present, but the latter effect does modify (6) since both  $c_m$  and  $R$  are affected by hindrance. The separate effect of hindrance can be extracted by the use of (3), and it can be shown that the rise of concentration during hindrance is relatively greater at the centre than at the corners.

On three occasions the restart of growth of a completely inert face was observed, and it was seen that there was then a sudden fall of concentration *at a single point*, not at a corner, on the face. From this point the change of concentration spread rapidly to either side, until, after about two seconds, the usual Bunn distribution was re-established.

### Discussion

Only one type of face, the (100), was investigated, and we have seen that this type, in  $\text{NaClO}_3$ , is capable of growing at the maximum rate permitted by diffusive presentation of material (for the Nernst condition amounts to this). It would be interesting to investigate other types of face by this optical technique, in order to see what values of concentration are in contact with such faces. At first sight it is puzzling how less stable types of face could succeed in obtaining the additional supply of material necessary for their higher rates of advance. This might be achieved by dendritic growth in which a face, in broad terms, does not 'wait for' the presentation of solute by diffusion. Or it might be found, despite thermodynamical objections, that less stable types of face have a lower solubility.

Regarding the 'hindrance' effect, there seems no doubt that this is caused by some process at the face itself. Previously the fact that simple (100)-bounded crystals rarely grow as perfect cubes has been ascribed to irregularities in the supply of material caused by imperfect stirring or by the presence of other crystals in the neighbourhood. Some more directly crystallographic explanation is now necessary. The three salient facts are, firstly, the discontinuous change from one degree of hindrance to another, secondly, the long periods, i.e., during the deposition of many new crystal planes, of its operation, and thirdly the rise of contiguous concentration accompanying it. The blockage of the nucleation point by a foreign adsorption is adequate as an explanation of the rarer event of complete stoppage of growth. It is probable that the observation mentioned at the end of the previous section represented either the restart of nucleation after ejection of an impurity or the start of nucleation at some new point. But explanations of hindrance in terms of impurities are not satisfactory. If impurities are sufficiently common to cause the very general hindrance effect, how could some faces remain unhindered, as they do, for long periods? Again, how could the impurity continue to have effect during the deposition of many new crystal planes?

The sudden changes point to some event, nevertheless, at the nucleation point; neither the discontinuity of change nor the selection of certain faces is consistent with a non-localized change on the face, say, in its topography or in a Volmer adsorbed phase. But no facile explanation comes to mind, and the effect must remain temporarily obscure and open to discussion.

Turning to the non-uniformity of concentration and normal gradient along the face, the author favours the acceptance of an intermediate layer, perhaps a Volmer phase, with properties dependent on position on the face. Although a flow in such a layer has not, perhaps, been conclusively demonstrated, it is reasonable to postulate its existence. It could well arise by interaction between the layer and the advancing growth sheets of the face, and non-uniformity of density, or perhaps concentration, would be expected in it which would give rise to non-uniformity in the concentration of the external solution contiguous to it and to non-uniformity of intake from outside. If desired, part of the non-uniformity in the layer could be ascribed

to the existence of vicinal planes on the underlying surface. For Volmer has said that the surface free energy of the underlying surface would affect the density of his phase. But, as said in the previous section, it is unlikely that surface topography alone could account for the large contiguous non-uniformities observed.

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## THE LINEAR VELOCITY OF POLYMORPHIC TRANSFORMATIONS

BY N. H. HARTSHORNE

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In an earlier paper<sup>1</sup> on the transformation of  $\alpha$ - to  $\beta$ -o-nitroaniline it was suggested that the linear rate of advance of the interface was determined by the difference between the rates of escape of molecules from the two crystal lattices, these rates being assumed to be the same, or at least to have the same dependence on temperature, as the rates of evaporation of the crystals into a vacuum. This picture, involving molecules travelling in both directions across the interface instead of only from the unstable to the stable lattice, seemed to be demanded by general kinetic considerations such as are applied to other types of phase boundaries. The suggestion of equality between the rates of escape and rates of evaporation into a vacuum arose from the fact that the apparent activation energy of the transformation (i.e., the value deduced from the slope of the graph of log rate against  $1/T$ ) was found to be of the same order as the internal latent heat of sublimation of the  $\beta$ -form.

In transformations studied later (yellow to red mercuric iodide,<sup>2</sup> monoclinic to rhombic sulphur<sup>3</sup>), the apparent activation energy was found to be less than the heat of sublimation (though very much greater than the heat of fusion), suggesting that in these cases the energy required to surmount the potential barrier at the interface was less than that involved in evaporation into a free vapour space. (A more recent study of the sulphur transformation, to be described later in this paper, has cast doubt on this interpretation as far as this substance is concerned.)

The form of the expression giving the rate of escape of molecules from a lattice will not be affected by the question as to whether the activation energy is equal to or less than the heat of sublimation. In either case the probability of a molecule acquiring this energy will be given to a close approximation by the simple exponential factor  $e^{-E/RT}$ . We can therefore proceed to derive a general expression for the linear rate on the basis of the above ideas as follows.

Let it be assumed that there exists at the interface between the two lattices a thin transitional layer of the order of one molecule in thickness, composed of molecules of high energy in a state of disorder. Molecules escape from each lattice into this layer as they acquire sufficient energy.

<sup>1</sup> Hartshorne, Walters and Williams, *J. Chem. Soc.*, 1935, 1860.

<sup>2</sup> Eade and Hartshorne, *J. Chem. Soc.*, 1938, 1636.

<sup>3</sup> Elias, Hartshorne and James, *J. Chem. Soc.*, 1940, 588.



They then stand an equal chance of either returning to their parent lattice or of condensing on the opposite lattice, i.e., the probability that a molecule which breaks free from one crystal modification will contribute to the growth of the other is  $\frac{1}{2}$ .

Consider first the transformation of an enantiotropic substance below the transition point. If  $v_a$  is the rate of escape of molecules from the unstable form, and  $v_\beta$  that from the stable form, both multiplied by the appropriate factor to convert them to linear rates of recession of the crystal surfaces, we have that  $V$ , the linear rate of advance of the interface, is given by

$$\begin{aligned} V &= \frac{1}{2}(v_a - v_\beta) \\ &= \frac{1}{2}(A_a e^{-E_a/RT} - A_\beta e^{-E_\beta/RT}) \end{aligned} \quad (1)$$

where  $E_a$  and  $E_\beta$  are the activation energies of escape, and  $A_a$  and  $A_\beta$  are factors which depend on the vibration frequencies of the molecules in the crystals and which to a first approximation may be taken as independent of temperature.

Now  $E_\beta = E_a + q$ ,

where  $q$  is the heat of transformation. Substituting for  $E_\beta$  in (1), we obtain

$$V = \frac{1}{2}e^{-E_a/RT}(A_a - A_\beta e^{-q/RT}) \quad (2)$$

But at the transition point,  $T_o$ ,  $V = 0$ . Therefore

$$\begin{aligned} A_a &= A_\beta e^{-q/RT_o}, \\ \text{or } A_\beta &= A_a e^{q/RT_o}. \end{aligned}$$

Substituting for  $A_\beta$  in (2), we obtain

$$V = \frac{1}{2}A_a e^{-E_a/RT} \left( 1 - e^{\frac{q}{R} \left( \frac{1}{T_o} - \frac{1}{T} \right)} \right) \quad (3)^*$$

It is assumed that  $q$ ,  $E_a$ , and  $E_\beta$  are independent of temperature.

According to this equation,  $V$  passes through a maximum value, as is observed in practice. We may obtain a value for the temperature,  $T_{\max.}$ , at which the velocity has this maximum value by differentiating  $V$  with respect to  $T$  and equating to zero, whence

$$T_{\max.} = 1 / \left\{ \frac{1}{T_o} + \frac{R}{q} \ln \left( 1 + \frac{q}{E_a} \right) \right\} \quad (4)$$

Now if  $E_a$  approaches the value of the heat of sublimation, the ratio  $q/E_a$  will, in general, be small, since the heats of transformation of polymorphs are usually small. Thus

$$\ln \left( 1 + \frac{q}{E_a} \right) \approx \frac{q}{E_a},$$

whence

$$T_{\max.} \approx 1 / \left( \frac{1}{T_o} + \frac{R}{E_a} \right) \quad (5)$$

Within this approximation, therefore, the interval between  $T_o$  and  $T_{\max.}$  increases as  $E_a$  decreases, and is practically independent of the value of  $q$ .

Eqn. (3) may be put in the following logarithmic form:

$$\ln V - \ln \left( 1 - e^{\frac{q}{R} \left( \frac{1}{T_o} - \frac{1}{T} \right)} \right) = - \frac{E_a}{RT} + \ln \frac{A_a}{2} \quad (6)$$

From this it is seen that the plot of the difference between  $\ln V$  and  $\ln \left( 1 - e^{\frac{q}{R} \left( \frac{1}{T_o} - \frac{1}{T} \right)} \right)$  is a straight line with a slope of  $-E_a/R$ . In addition;

\* This expression in a slightly different form was first deduced by the author in 1938, and in its present form in 1942. It has not previously been published because until recently there has seemed to be no trustworthy data covering a sufficiently wide range of temperature by which it could be tested.

inspection of the equation shows that the plot of  $\ln V$  against  $1/T$ , at temperatures below  $T_{\max.}$ , will have a slope which tends more and more nearly to  $-E_a/R$  as  $T$  decreases. For example, suppose that  $E_a = 22,500$  cal.,  $q = 730$  cal., and  $T_0 = 369^\circ \text{K}$ , whence  $T_{\max.}$  (from (5)) is  $354^\circ \text{K}$ . The mean slope of the graph of  $\ln V$  against  $1/T$  then corresponds to the following  $E$  values (apparent activation energies) for the temperature ranges given:  $313 - 293^\circ \text{K}$ , 19,400 cal.;  $293 - 273^\circ \text{K}$ , 20,500 cal.;  $273 - 253^\circ \text{K}$ , 21,100 cal. These figures show that, provided that eqn. (3) is valid, the apparent activation energy for a temperature range sufficiently far below  $T_{\max.}$  (say,  $50^\circ$  or more) may be taken as an indication of the order of the true activation energy,  $E_a$ , for the process of transfer of molecules from the unstable to the stable lattice. This will also apply in general to the apparent activation energy in monotropic transformations, such as the  $\alpha$ - to  $\beta$ - change in *o*-nitroaniline mentioned above, for in these cases the (theoretical) transition point lies above the melting point, and  $T_{\max.}$  also may be expected to be in this region.

Eqn. (3) may be modified to apply to an enantiotropic transformation above the transition point simply by changing the sign of the quantity in the brackets, thus :

$$V' = \frac{1}{2} A_\alpha e^{-E_a/RT} \left( e^{\frac{q}{R} \left( \frac{1}{T_0} - \frac{1}{T} \right)} - 1 \right) \quad (7)$$

The subscript  $\alpha$  still refers to the form which is unstable below the transition point, now the *stable* form, and  $T$  is now greater than  $T_0$ . The equation corresponds to a continuous increase of the linear rate  $V'$  with rise of temperature.

By expanding the exponential term inside the brackets in eqn. (3) and neglecting all but the first two terms of the expansion, we obtain

$$V = \frac{1}{2} A_\alpha \cdot \frac{q}{RT} \left( \frac{T_0 - T}{T_0} \right) e^{-E_a/RT} \quad (8)$$

This approximation is a close one only if

$$\frac{q}{RT} \left( \frac{T_0 - T}{T_0} \right)$$

is a small fraction, i.e., if  $q$  is small and  $T$  is not too far below  $T_0$ . From the general thermodynamic relation  $G = H - TS$ , where  $G$  is the free energy,  $H$  the heat content, and  $S$  the entropy, it follows, since we have assumed that  $q$  ( $= -\Delta H$ ) is constant, that

$$q \left( \frac{T_0 - T}{T_0} \right) = -\Delta G.$$

Substituting in (8) we obtain

$$V = \frac{1}{2} A_\alpha \frac{(-\Delta G)}{RT} \cdot e^{-E_a/RT} \quad (9)$$

This form of the velocity equation is interesting in bringing out the influence of the difference between the free energies of the two modifications as the "driving force" of the reaction.

Equations similar to (8) and (9) have been derived independently by Akulov<sup>4</sup> and by Laurent.<sup>5</sup> Akulov's argument is briefly as follows. He expresses the work done by a molecule in moving from one side of the interface to the other as  $\eta v \delta$ , where  $\eta$  is the coefficient of the internal resistance (arising from the collisions suffered by the molecule in its passage through the boundary layer),  $v$  is the average velocity of the molecule, and  $\delta$  the

<sup>4</sup> Akulov, *Compt. rend. U.R.S.S.*, 1941, **32**, 340; 1943, **39**, 268.

<sup>5</sup> Laurent, *Rev. Metall.*, 1945, **42**, 22.

average path covered. This work is equated to the difference between the free energies of the molecule in its initial and final positions. Thus

$$v = \frac{q}{\eta\delta} \left( \frac{T_0 - T}{T_0} \right)$$

where  $q$ ,  $T_0$  and  $T$  have the same significance as above. In addition, it is necessary that a molecule shall possess sufficient kinetic energy to surmount the potential barrier at the interface, and the probability of this is  $e^{-E/RT}$ , where  $E$  is the height of the barrier, or in the general case a certain function of this. The number of molecules crossing unit area of the interface in unit time, and therefore the linear rate, will be proportional to

$$v e^{-E/RT} \quad \text{or} \quad \frac{q}{\eta\delta} \left( \frac{T_0 - T}{T_0} \right) e^{-E/RT}.$$

Laurent has used very similar arguments to those on which eqn. (3) is based, the main differences being that he expresses the activation energy in terms of quantum theory, the molecules in the lattice being assumed to behave as simple harmonic oscillators, and that, despite this refinement, he adopts the same approximation as we have used here to obtain eqn. (8) and (9). His equation may be put in the form—

$$\text{Linear rate} = A \frac{(-\Delta G)}{RT} \cdot e^{\frac{1}{2} \left( \frac{\theta}{2} - \frac{E}{R} \right)},$$

where  $A$  is a constant,  $\theta = \frac{h\nu}{k}$ , the Einstein characteristic temperature, and  $E$  is the height of the potential barrier. Since  $\theta/2T$  is small compared with  $-E/RT$ , the exponential factor will be of the same order as  $e^{-E/RT}$ .

TABLE I

Temp. (° C)	Mean linear rate, $V$ (mm./hr.)	Standard deviation (S)	Coefficient of variation (S/V)
0	0.029	0.007	0.24
10	0.130	0.032	0.25
20	0.36*	0.10	0.28
30	0.86*	0.27	0.32
	0.94	0.17	0.18
40	1.98*	0.73	0.37
50	3.60	0.97	0.27
60	5.16	1.47	0.28
70	5.30	1.56	0.29
80	1.60	0.60	0.37

\* Results of Elias, Hartshorne and James.

The author, with M. H. Roberts, has now completed measurements of the linear rate of transformation of monoclinic to rhombic sulphur over a sufficiently wide range of temperatures (0° to 80° C) for eqn. (3) to be tested. Polycrystalline films of monoclinic sulphur between glass surfaces, prepared under controlled conditions and 0.06 to 0.1 mm. thick, were maintained at constant temperature, and after the reaction had been started by inoculating the edge of the film with rhombic sulphur, the position of the interface was observed at fixed intervals of time by projecting an enlarged image of the film on to a grid drawn on a white screen. The time taken for the interface to traverse each rectangle of the grid (corresponding to an advance of 0.25 mm.) was noted, about 1200 such readings being taken at each

temperature. From these readings the average rate, standard deviation, and coefficient of variation were calculated. This is similar to the method used by Elias, Hartshorne and James (loc. cit.), whose work, interrupted by the war, only covered the range 20° to 40° C. Somewhat different methods were used at 10° and 0°, where the interface movement was too slow to be conveniently followed in this way. The work will be fully described elsewhere.

The results, together with those of Elias, Hartshorne and James, are given in Table I, and in Fig. 1 (curve A),  $\log V$  is plotted against  $1/T$ . The short strokes above and below each point on the graph represent the standard deviation. The variance is considerable, but the means lie very nearly on a smooth curve, which shows a maximum at about 65° ( $T_0 = 95.6^\circ$ ). It is

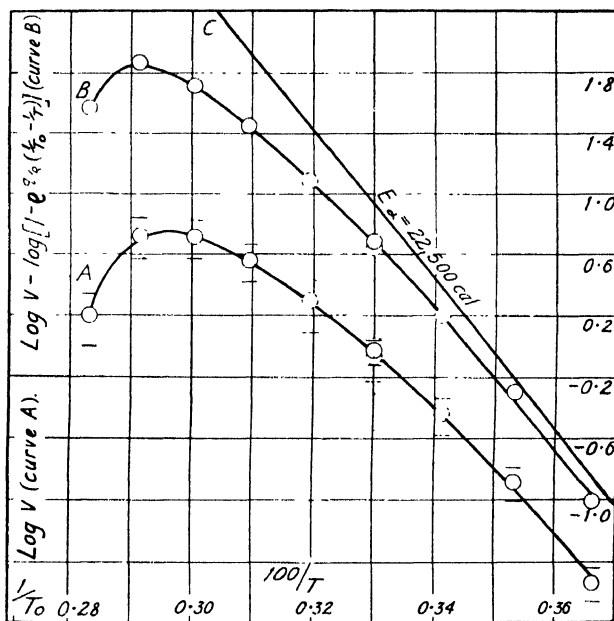


FIG. 1.—Results of Elias, Hartshorne and James indicated by broken lines.

particularly satisfactory that the earlier results, which were obtained with a different sample of sulphur and using a different apparatus, agree well with the later ones. It will also be seen that the coefficients of variation (Table I) remain fairly constant throughout the whole range, which may perhaps be taken to indicate that there is no essential change in the character of the reaction with change of temperature.

The third column of Table II gives the values of  $\log V - \log \left( 1 - e^{\frac{q}{T_0 - T}} \right)$  (which we will now write as  $\log \left( \frac{V}{1 - x} \right)$  for brevity), taking  $q$  to be 730 cal./mol. ( $S_8$ ). This is the mean, to the nearest 10 cal. of Brönsted's value of 616 cal. at 0°, determined directly<sup>6</sup> and that calculated by Neumann<sup>7</sup> for the transition temperature from measurements of the vapour pressures of the two modifications, namely, 840 cal. The change of  $q$  with temperature

<sup>6</sup> Brönsted, *Z. physik. Chem.*, 1906, **55**, 371.

<sup>7</sup> Neumann, *Z. physik. Chem.*, 1934, **171**, 416.

represented by these figures agrees well with that calculated from heat capacity data.<sup>8</sup> In Fig. 1,  $\log \left( \frac{V}{1-x} \right)$  is shown plotted against  $1/T$  (curve B). The plot is not a straight line as demanded by eqn. (6), and at first sight the deviation from this requirement seems very great, for the curve passes through a maximum. Similar deviations are found when the equations of Akulov and Laurent (above), put into the logarithmic form, are applied. It must be noted, however, that the value of  $(1-x)$  in the higher temperature range ( $70^\circ$  to  $80^\circ$ ) is extremely sensitive to small changes in  $x$ . An increase in  $x$  of only a few per cent. in this region is sufficient to abolish the maximum in the curve completely since it sharply elevates the values of  $\log \left( \frac{V}{1-x} \right)$ . On the other hand, a similar change in the lower temperature region has a comparatively small effect on the values of  $\log \left( \frac{V}{1-x} \right)$ , and practically none on the slope of the curve. It is thus possible by making appropriate

TABLE II  
 $q = 730 \text{ cal./mol.}$

Temp. (°C)	$(1-x)^*$	$\log \left( \frac{V}{1-x} \right)$	$E_a = 22,500 \text{ cal./mol.}$		
			$\varphi_{25.5} = 1.05$	$\varphi_{25.5} = 1.10$	$\varphi_{25.5} = 1.15$
0	0.295	-1.010	1.02	1.11	1.21
10	0.260	-0.301	0.98	1.07	1.16
20	0.227	0.203†	1.04	1.10	1.16
30	0.194	0.644†	1.08	1.12	1.16
		0.684	1.07	1.11	1.15
40	0.162	1.086†	1.08	1.11	1.14
50	0.131	1.437	1.09	1.10	1.12
60	0.101	1.708	1.08	1.09	1.10
70	0.071	1.871	1.07	1.07	1.07
80	0.043	1.571	1.04	1.04	1.04

$$* (1-x) = 1 - e^{\frac{q}{R} \left( \frac{1}{T} \right)}$$

† Results of Elias, Hartshorne and James.

but quite minor adjustments to the value of  $x$  to convert curve B into a straight line. It has therefore seemed worth while to consider whether there is any factor, not taken into account in deriving eqn. (6), which would act so as to increase slightly the values of  $x$ . A possibility is suggested by the marked difference which is observed in the appearance of the interface under the microscope at the lower temperatures from that at temperatures around and above  $T_{\max}$ . In the former case the advancing front of the rhombic phase is finely serrated, indicating that the particle size is small; in the latter it consists of quite large crystals with well-developed faces. We may suppose that at the lowest temperatures the rhombic phase forms as a finely divided mass which does not recrystallize, or only very slowly. Rise of temperature will favour recrystallization, but up to the region of  $T_{\max}$  this will be offset, as far as conditions at the actual interface are concerned, by the increase in the linear rate, which will act so that recrystallization will be effectively confined to the material *in rear* of the advancing

<sup>8</sup> Lewis and Randall, *J. Amer. Chem. Soc.*, 1914, **36**, 2468.

front. Above  $T_{\max.}$ , however, the linear rate progressively slackens and with rise of temperature conditions will become more and more favourable for the formation of large, well-ordered crystal planes at the interface itself. We may further suppose that owing to the fine state of division at temperatures up to  $T_{\max.}$ , the rate of escape of molecules from the rhombic form into the transition layer will be greater than the normal value for large crystals because of increased surface energy, but that above  $T_{\max.}$  the rate of escape will tend towards the normal value as the transition point,  $T_0$ , is approached. On this view, eqn. (1) becomes

$$V = \frac{1}{2}(A_\alpha e^{-E_\alpha/RT} - \phi \cdot A_\beta e^{-E_\beta/RT}),$$

where  $\phi$  is greater than unity and is approximately constant up to  $T_{\max.}$ , and then decreases to become unity at  $T_0$ . From this we obtain the equation:

$$\ln\left(\frac{V}{1 - \phi x}\right) = -\frac{E_\alpha}{RT} + \ln \frac{A_\alpha}{2} \quad (10)$$

Since, as stated above, small increases in  $x$  make very little difference to the slope of curve B at the low temperature end, we may use this part of the curve to obtain a value for  $E_\alpha$ , and in this way we find that it lies between 22,000 and 23,000 cal./mol. Now the internal latent heat of sublimation of monoclinic sulphur as given by Neumann's vapour pressure results (loc. cit.) is 22,500 cal./mol. to the nearest 100 cal. The slope of line c in Fig. 1 corresponds to this value. It thus appears that  $E_\alpha$  is the same, or nearly the same, as the energy which the molecules must acquire to escape completely from the monoclinic lattice into a free vapour space. Taking it to be 22,500 cal., we may test the applicability of eqn. (10). Bearing in mind the argument on which this equation is based, the problem is to see whether it is possible to find a value for  $\phi$ , not much greater than unity, which is constant over the range from  $0^\circ$  to the region of  $T_{\max.}$ , and which gives a straight line plot of  $\log\left(\frac{V}{1 - \phi x}\right)$  against  $1/T$  with a slope corresponding to 22,500 cal. The method which has been adopted is to assume different values of  $\phi$  for a temperature of  $25.5^\circ$  which lies midway between  $0^\circ$  and  $T_{\max.}$  on the reciprocal scale, and then work out values for other temperatures on the assumption that the above linear relationship holds. The last three columns of Table II show the results obtained taking  $\phi$  at  $25.5^\circ$  as 1.05, 1.10 and 1.15 respectively. It will be seen that up to  $50^\circ$  there is an upward trend in the first case, a downward one in the last, but that at 1.10 the values show no trend. (The variation from strict constancy may be attributed to errors in the value of  $V$ . If we reverse the calculation by assuming a constant value of 1.10 for  $\phi$ , and work out the corresponding values of  $V$ , we obtain results which are very close to the experimental ones, and well inside the standard deviation limits for these.) From  $60^\circ$  onwards the  $\phi$  values decline in all cases. The requirements of the theory are thus satisfied by  $\phi = 1.10$ , and this is a not improbable figure.

The real meaning of the  $\phi$  factor is that the *activation energy* for the escape of molecules from the rhombic form is reduced below the normal value applicable to large crystals. Thus we may write:

$$\phi e^{-E_\beta/RT} = e^{-E'_\beta/RT},$$

where  $E'_\beta$  is this reduced activation energy. For  $\phi = 1.10$  and  $E_\beta = 23,230$  cal.  $= (E_\alpha + q)$ ,  $E'_\beta$  has the mean value of 23,173 cal. for the range  $0^\circ$  to  $50^\circ$ , which is only 57 cal., or 0.25 %, less than  $E_\beta$ . This corresponds to an increase of 57 cal. in  $q$  as the temperature rises from  $50^\circ$  to  $T_0$ . In the

light of this we can say that the deviations of curve B from the requirements of (6), if expressed as percentage variations of  $E_\beta$ , are quite small.

From eqn. (10), the temperature-independent factor  $A_\alpha$  may be calculated. Taking  $E_\alpha$  as 22,500 cal. and  $\phi$  in the lower temperature range as 1.10, the value obtained is  $2.0 \times 10^{17}$  mm./hr. or  $0.56 \times 10^{13}$  cm./sec. This is greater than the speed of light. An even higher factor ( $10^{14}$  to  $10^{16}$  cm./sec.) was obtained by Anderson and Mehl<sup>9</sup> from measurements of the linear rate of recrystallization of cold-rolled aluminium. Burgers<sup>10</sup> and N. F. Mott have suggested that this very high value indicates that some process which depends on temperature through an exponential factor  $e^{-E/RT}$  triggers the change of crystal form of a whole mosaic block without the intervention of any other thermally activated process, though Mott has also given an alternative explanation.<sup>11</sup> On the basis of this theory we may equate  $A_\alpha$  to  $Bvd$ , where  $v$  is the vibration frequency of the molecules, and  $d$  the average spacing between them, in the monoclinic crystal, and  $B$  is the number of molecules whose rearrangement is initiated by the thermal activation of one molecule. Taking  $v$  as  $10^{12}$  —  $10^{13}$ , and  $d$  (from the density) as  $6 \times 10^{-8}$  cm.,  $B$  works out to the order of  $10^7$ . The volume occupied by this number of molecules in monoclinic sulphur is about  $10^{-15}$  cm.<sup>3</sup>, which is within the range of the usual estimates of the size of a mosaic block.

In showing that the deviations of the experimental results from eqn. (6) can be accounted for by means of the  $\phi$  factor, it has not been forgotten that this equation was deduced on the assumption that  $A_\alpha$ ,  $A_\beta$ ,  $E_\alpha$ ,  $E_\beta$ , and therefore  $q$ , were all independent of temperature, whereas at best this can only be an approximation. The increase of  $q$  with temperature given by the results of Brönsted and Neumann, and calculated from the heat capacities recorded by Lewis and Randall (see above), is in fact greater than that corresponding to the introduction of the  $\phi$  factor, and this suggests that the deviations may be due to the approximations inherent in the equation rather than to changes in surface energy. We cannot, of course, consider this possibility solely on the basis of the thermodynamic variation of  $q$ , i.e., without regard to the temperature dependence of the other four 'constants,' and unfortunately  $q$  appears to be the only one whose temperature dependence can be assessed with any certainty. It is, however, intended to look further into this question. It has also been suggested to the author by Prof. E. G. Cox that the average size of the mosaic blocks of the monoclinic phase may vary with temperature as a result of the way in which the films are prepared. This would result in corresponding changes in  $A_\alpha$ , if the Burgers-Mott trigger mechanism be accepted.

ADDENDUM. (Received 13th May, 1949.)

Since the above paper was presented, it has been pointed out to the author by Dr. W. J. Dunning that the temperature coefficient of the reaction can be accounted for on the basis of Volmer's equation for the linear rate of growth of a crystal from its vapour.<sup>12</sup> Applied to a solid-solid reaction Dunning's treatment assumes that the transitional layer between the two lattices behaves as a true vapour (in which case it will have to be somewhat thicker than one molecule) and that the rate of advance of the interface depends not only on the supersaturation of the vapour with respect to the stable phase, but also on the probability of formation of two-dimensional nuclei on the completed surface planes of molecules of this phase.

<sup>9</sup> Anderson and Mehl, *Trans. Amer. Inst. Min. Met. Eng.*, 1945, Tech. Pub. No. 1805.

<sup>10</sup> Burgers, *K. Ned. Ak. Wet.*, 1947, 50, 719.

<sup>11</sup> Mott, *Proc. Physic. Soc.*, 1948, 60, 391.

<sup>12</sup> Volmer, *Kinetik der Phasenbildung*, 1939, p. 174.

For this case Volmer's equation can be put in the simplified form :

$$V = K \cdot e^{-E/RT} \cdot e^{-A''/RT}, \quad (11)$$

where  $E$  is the activation energy for the escape of molecules from the unstable lattice (i.e., the internal latent heat of sublimation), and  $A''$  is that for the formation of two-dimensional nuclei. Now

$$\frac{A''}{RT} = \frac{\omega M \rho^2 N T_0}{2q\delta\delta RT(T_0 - T)} = \frac{\text{const.}}{T(T_0 - T)}, \quad (12)$$

where  $\omega$  is a shape factor,  $M$  is the molecular weight,  $\rho$  the edge free energy,  $N$  the Avogadro number,  $q$  the heat of transformation,  $\delta$  the density,  $\delta$  the spacing between lattice planes and the other symbols have the usual significance. Substituting for  $A''/RT$  and taking logarithms, eqn. (11) becomes:

$$\ln V = \ln K - \frac{E}{RT} - \frac{\text{const.}}{T(T_0 - T)}. \quad (13)$$

Dunning finds that this equation fits the results given in Table I very well, when  $\ln K = 38.77$ ,  $E = 20,200$  cal., and the constant in the third term is  $3.5 \times 10^4$ . ( $K$  is of the same order of magnitude as the author's  $A_\alpha$ , so that this treatment does not throw any light on the reason for the large pre-exponential factor.)

This contribution to the problem is most interesting and important, and the possibility that surface nucleation is a rate-determining factor will receive serious consideration in future work carried out by the author on solid-solid transformations. For the present, however, the following objections to the theory, at least in its present form, and as applied to the case of sulphur, may be raised.

(1) The marked difference, mentioned in the paper, between the surface contour of the rhombic phase above and below  $T_{\text{max}}$ , suggests strongly that if surface nucleation is necessary at all, it is not equally so over the whole range of temperature; that is to say, the effect cannot be expressed by the simple factor  $e^{-A''/RT}$  as defined above. At temperatures below  $50^\circ$  the advancing front exhibits numerous *rounded* promontories of the order of  $10^{-4}$  cm. or less in diameter, and it is difficult to reconcile these with the Volmer picture of the successive laying down of extended plane layers of molecules. Possibly surface nucleation only becomes necessary at the higher temperatures, and if so, this could account for the negative deviations from eqn. (3) in this region.

(2) R. S. Bradley<sup>13</sup> has found from measurements of the rate of evaporation of single crystals of rhombic sulphur between  $15^\circ$  and  $32.5^\circ$  C that the accommodation coefficient, i.e., the fraction of molecules which on striking the surface condense, is constant at 0.7. This could be interpreted as indicating that an activation energy of about 200 cal. is required for surface nucleation (by equating 0.7 to  $e^{-A''/RT}$ ), but this is very much less than the value calculated from eqn. (12) for this temperature range, viz., 700–1000 cal. Alternatively the deviation of the coefficient from unity may be due to an orientation requirement, i.e., that molecules approaching the surface must have orientations within a certain solid angle if they are to condense.

(3) The value  $3.5 \times 10^4$  for the constant in the third term of eqn. (13) corresponds to an edge free energy ( $\rho$ ) of  $2.4 \times 10^{-7}$  erg/cm. (taking  $\omega$  as  $2\pi$ ), from which the surface free energy may be calculated to be of the order of 4 ergs/cm.<sup>2</sup>. Bearing in mind that, according to the theory, the growing surface is supposed to be in contact with vapour, this value is far too small. The surface free energy of liquid sulphur just above the melting point is

<sup>13</sup> Private communication.



60 ergs/cm.<sup>2</sup> and that for the solid must be somewhat greater. If the  $\rho$  value corresponding to this is inserted in eqn. (13), the constant is increased by a factor of about 200, and the equation no longer fits the experimental results.

(4) The picture of a fairly thick transitional layer of vapour between the two lattices introduces difficulties. Owing to the increase of density accompanying the transformation, the vapour gap would progressively widen as the reaction proceeded, and it can be shown that this would result in a concomitant decline of the linear rate. Such a decline was in fact observed by the author with *o*-nitroaniline and mercuric iodide (*loc. cit.*) and the theory of a progressively widening gap was invoked to explain it. In the case of sulphur, however, the rate is constant at constant temperature. This is probably due to the fact that, as can be seen under the microscope, the shrinkage accompanying the transformation is continuously accommodated by the formation of short cracks at, or just behind, the interface. This shows that solid-solid contact is never completely lost.

Against all this, however, must be set the fact that both by Dunning's and the author's treatments the activation energy for escape of molecules comes out to be of the same order as the heat of sublimation. Further, if the linear rate is calculated on the assumption that it is given by the difference between the rates of evaporation of the two forms (using the

usual equation,  $v = \alpha p \frac{1}{\sqrt{2\pi MRT}}$ ), the result is of the order of  $10^{-2}$  to  $10^{-3}$  of the observed rate, i.e., the discrepancy between theory and observation is much less than when, as in the paper, the temperature independent factor is expressed as the product of the vibration frequency and the lattice spacing.

From the above discussion it is very evident that much remains to be discovered about the mechanism of solid-solid transformations.

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## BOUNDARY MIGRATION AND GRAIN GROWTH \*

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It has long been known that metals will show grain growth and that this growth involves a reorientation of metal atoms across grain boundaries in such a way that many grains disappear entirely. This movement of

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*a*



*b*

FIG. 1.—TNT during boundary migration at 80° C (*a* after 1 min.; *b* after 4 min.) ( $\times 100$ ) crossed Nicols.



*a*



*b*

FIG. 2.—DDT showing secondary crystallization due to boundary migration (*b* is an enlargement ( $\times 100$ ) of part of *a*). ( $\times 40$ ) crossed Nicols.

grain boundaries led to the term "boundary migration"<sup>1</sup> which will be used here as a synonym for grain growth.

In 1929 Tammann<sup>2</sup> published data showing that certain compounds (camphor, pinene hydrochloride and ice) show a behaviour very similar to that observed in metals. In 1946 Buerger and Washken<sup>3</sup> showed that some minerals (e.g., anhydrite, fluorite, periclase and corundum) when compressed and heated to temperatures well below the melting point would also show boundary migration similar to metals. In 1949 the study of octachloropropane was suggested<sup>4</sup> as a means of studying boundary migration in metals.

During the past several years a number of organic compounds quite dissimilar to octachloropropane in lattice properties have been shown to exhibit boundary migration. For example, Kofler<sup>5</sup> reported in 1941 that an organic compound, TNT, shows a somewhat similar behaviour in that crystals once formed undergo a further recrystallization in the solid phase so that one crystal grows into and through its neighbour (Fig. 1). DDT has been reported<sup>6</sup> and several other organic compounds (unreported) have been observed to show similar behaviour (Fig. 2). In each of these cases and in contrast with the metals, camphor, fluorite, octachloropropane, etc., it is apparent that these materials show boundary migration in which direction is dependent on the orientation of the crystal lattice within the grains.

Metals, octachloropropane, camphor, pinene hydrochloride, ice, fluorite, anhydrite, etc., show migration of one crystal into another in such a way that the orientation of the lattice cannot be an important factor. On the other hand, boundary migration by TNT, DDT, Vitamin K, etc., is definitely dependent on orientation of the crystals. The crystals will grow in a direction which can be predicted for a given compound from the known relative orientations.

Two different types of boundary migration are therefore recognized. The two types will be described throughout as the DDT type, in which orientation controls the direction of boundary migration; and the octachloropropane type, in which orientation has little or no effect on the direction of boundary migration.

The DDT type of boundary migration is of particular interest since as stated above the direction of growth is dependent on lattice orientation. Any theory covering the mechanism of boundary migration must take into account, for crystals of this type, the effect of difference in orientation of the two lattices in contact. DDT, for example, grows in such a way that the (001) face will penetrate either the (100) or (010) planes of adjacent crystals. If, on the other hand, crystals of this type are aligned parallel to each other no growth will occur. Maximum growth will occur, therefore, when crystals elongated parallel to *c* intersect at 90° angles (Fig. 2).

TNT shows a very similar behaviour although it does not grow as rapidly during boundary migration. It does, however, grow in much the same manner and in such a way that the direction of migration can always be predicted from the orientation of the crystals. In this case the (010) face will always grow into the (001) and (100) faces (Fig. 1).

During the past 20 or 30 years there has been considerable discussion regarding the possible mechanism by which boundary migration occurs.

<sup>1</sup> Carpenter and Elam, *J. Inst. Metals*, 1920, **24**, 123.

<sup>2</sup> Tammann, *Z. anorg. Chem.*, 1929, **182**, 289.

<sup>3</sup> Buerger and Washken, *Amer. Miner.*, 1947, **32**, 296.

<sup>4</sup> McCrone, *J. Appl. Physics*, 1949, **20** (Feb.).

<sup>5</sup> Kofler, *Z. physik. Chem. A*, 1941, **188**, 201.

<sup>6</sup> McCrone, *Anal. Chem.*, 1948, **20**, 274.

Most of this discussion has been on boundary migration of the octachloropropane type and most of it has concerned metals. Harker and Parker<sup>7</sup> have advanced the argument that grain shape governs the extent and direction of boundary migration. This results in movement of the grain boundaries in such a way that straight boundaries meet at angles of  $120^\circ$ . By this criterion little or no grain growth should occur when these conditions are satisfied. The effect of lattice deformation on boundary migration is not discussed by them, although presumably it would at least affect the angles between grain boundaries. Most other investigators have assumed that strain energy, due to cold-working and resultant plastic deformation, is the driving force.

Two hypothetical questions can be posed as a result of irreconcilable of these two ideas—

1. Can grain growth occur in a sample whose grains meet throughout at  $120^\circ$  angles with straight boundaries but in which the grains possess residual strain energy?

2. Can grain growth occur in a sample whose grains show curved boundaries and many angles not equal to  $120^\circ$  but in which the grains are strain-free?

Unfortunately the first of these questions cannot be answered in an unequivocal fashion. A close approximation to a final answer to the second can, however, be obtained. This is done by comparing the rate of growth in two samples: one with, and the other as nearly as possible without, strain. Experimental data to answer this question are presented below.

A broader problem, however, and one of great interest and importance is to find a more definite relation between boundary migration in metals and in the octachloropropane type of organic compound. It is obvious on examination of photomicrographs showing boundary migration in systems of these two kinds that in superficial appearance there is no difference between the two cases. There is a striking similarity between growth in metals and in octachloropropane and the resulting structures are amazingly similar in appearance before, during and after boundary migration. Furthermore, octachloropropane and other organic compounds of this type show a final structure which agrees entirely with the ideas presented by Harker and Parker.<sup>7</sup> Octachloropropane, for example, during annealing changes progressively toward an ultimate appearance in which all grain boundaries are straight and meet only at angles of  $120^\circ$  (Fig. 3).

An additional effort has been made to relate boundary migration of octachloropropane to that of metals. This is being done by studying the rate of growth at different temperatures and comparing these data with corresponding data for metals systems. Unfortunately little data of the latter type are available and it appears very difficult to accumulate large amounts of such data because of the experimental difficulties. It is possible, on the other hand, to follow boundary migration in organic compounds during annealing of a thin transparent section using polarized light under controlled temperature conditions and to obtain a complete curve with as many experimental points as desirable in a few hours.

Some data taken in this way are summarized in Table I. These data were obtained by the following procedures.

Expt. 1-4: A small quantity (5-10 mg.) of octachloropropane (purified by sublimation to a melting point of  $168^\circ\text{C}$ ) was melted between a cover glass and slide. The fused preparation was quenched quickly to room temperature by placing it cover-glass side down on a metal block. This preparation was then placed in a previously heated hot-stage set at the desired temperature. About 10 sec. was required for the slide to become heated and from 30-60 sec. to find

<sup>7</sup> Harker and Parker, *Trans. Amer. Soc. Metals*, 1945, **34**, 156.

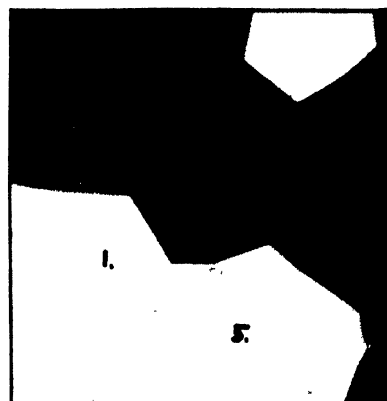
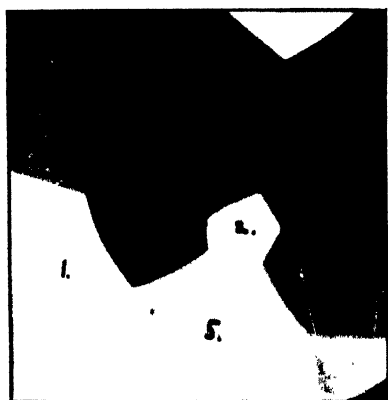
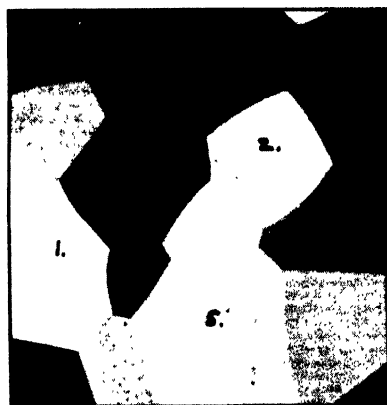


FIG. 3.—Boundary migration in octachloropropane, the numbers refer to the same crystal as it appears at successive times. ( $\times 100$ ) crossed Nicols.



TABLE I

## ISOTHERMAL TIME-RATE DATA FOR OCTACHLOROPROPANE

## Expt. 1 : 136° C

Time (min.)	Log Time	Rate	Log Rate	Diam. (micron)	Log Diam.
5	0.70	0.010	8.00-10	115	2.06
6	0.78	0.007	7.85-10	117	2.07
7	0.85	0.006	7.78-10	130	2.11
10	1.00	0.005	7.70-10	146	2.16
13	1.15	0.004	7.60-10	153	2.18
16	1.20	0.004	7.60-10	196	2.29
22	1.34	0.004	7.60-10	207	2.32
27	1.43	0.004	7.60-10	221	2.34
32	1.51	0.004	7.60-10	261	2.42
41	1.61	0.003	7.48-10	249	2.40
54	1.73	0.003	7.48-10	290	2.46
64	1.81	0.003	7.48-10	344	2.54

## Expt. 2 : 123° C

1	0.00	—	—	210	2.32
2	0.30	—	—	228	2.36
3	0.48	—	—	230	2.36
5	0.60	0.0022	7.34-10	236	2.37
7	0.85	—	—	238	2.38
10	1.00	0.0016	7.20-10	239	2.38
15	1.17	0.0015	7.18-10	247	2.39
28	1.45	0.0011	7.04-10	263	2.42
45	1.65	0.0010	7.00-10	278	2.44
80	1.90	0.0009	6.95-10	314	2.50
140	2.15	0.0008	6.90-10	377	2.58

## Expt. 3 : 115° C

1	0.00	0.0008	6.90-10	160	2.20
30	1.48	0.0007	6.85-10	192	2.28
60	1.78	0.00055	6.74-10	198	2.30
120	2.08	0.00035	6.54-10	232	2.37
180	2.26	0.00025	6.40-10	234	2.37
240	2.38	0.00020	6.30-10	257	2.41
300	2.48	0.00020	6.30-10	258	2.41

## Expt. 4 : 103° C

1	0.00	0.00056	6.75-10	193	2.29
10	1.00	0.00033	6.52-10	199	2.30
20	1.30	0.00022	6.34-10	204	2.31
1040	3.02	0.00013	5.11-10	241	2.38
1485	3.18	0.00013	5.11-10	265	2.42
2100	3.32	0.00013	5.11-10	258	2.41
3390	3.53	0.00013	5.11-10	283	2.45



TABLE I—(Continued)

Expt. 5: 136° C					
Time (min.)	Log Time	Rate	Log Rate	Diam. (micron)	Log Diam.
1	0.00	0.015	8.18-10	59	1.77
1.5	0.18	0.013	8.11-10	67	1.83
2.5	0.40	0.010	8.10-10	78	1.89
4.5	0.65	0.0065	7.81-10	115	2.06
5.5	0.74	0.0055	7.74-10	127	2.10
7.5	0.88	0.0050	7.70-10	134	2.13
10	1.00	0.0038	7.58-10	143	2.16
15	1.18	0.0038	7.58-10	151	2.18
27	1.43	0.0038	7.58-10	209	2.32
40	1.60	0.0038	7.58-10	261	2.42
60	1.78	0.0038	7.58-10	330	2.52
90	1.95	0.0038	7.58-10	356	2.55
120	2.08	0.0038	7.58-10	435	2.64
140	2.15	0.0038	7.58-10	638	2.80
160	2.20	0.0038	7.58-10	770	2.89

Expt. 6: 159° C					
4	0.60	0.006	7.78-10	185	2.27
9	0.95	0.006	7.78-10	188	2.27
13	1.11	0.006	7.78-10	204	2.31
18	1.26	0.006	7.78-10	231	2.36
23	1.36	0.006	7.78-10	287	2.46

Expt. 7: 145° C					
1	0.00	0.0008	6.90-10	171	2.23
40	1.60	0.0008	6.90-10	203	2.31

an appropriate field of view. In all experiments zero time indicates the time at which the preparation was placed in the hot-stage. Most of the readings were started at  $M = 1$  min.

A carefully calibrated Kofler hot-stage was used with a Sola constant voltage transformer. The temperature data are accurate to  $\pm 1^\circ\text{C}$  and accurately represent the temperature of the field under observation. The data were taken by means of photomicrography using a Leica with a Speed-O-Copy attachment. The 35 mm. negatives were enlarged to a convenient magnification and the average grain size was determined by measuring the intersections of grain boundaries on a linear scale during a number of regularly spaced linear traverses of the entire field (Fig. 3).

*Expt. 5:* A small quantity (5-10 mg.) of octachloropropane (purified as above) was placed between a slide and cover-glass and subjected to 500 psi pressure. This preparation was then placed in a previously heated hot-stage as for Expt. 1-4.

*Expt. 6 and 7:* In these two experiments 5-10 mg. of octachloropropane was melted in the usual way between a slide and cover-glass. The preparation was then, however, placed immediately in the previously heated hot-stage so that the temperature of the preparation at no time fell below  $145^\circ\text{C}$  (Expt. 7) or  $159^\circ\text{C}$  (Expt. 6).

The average diameters were then determined in the same manner as described above. Fig. 4 shows these average diameters as a function of time for each experiment. These data were then smoothed from these curves and rate of growth data were calculated from the slopes of these smoothed curves. Fig. 5 shows log rate against log time for each experiment. Fig. 6 shows log rate against temperature with a vertical line for each experiment covering the time variable. The actual data points fall on the vertical lines with increasing time downward.

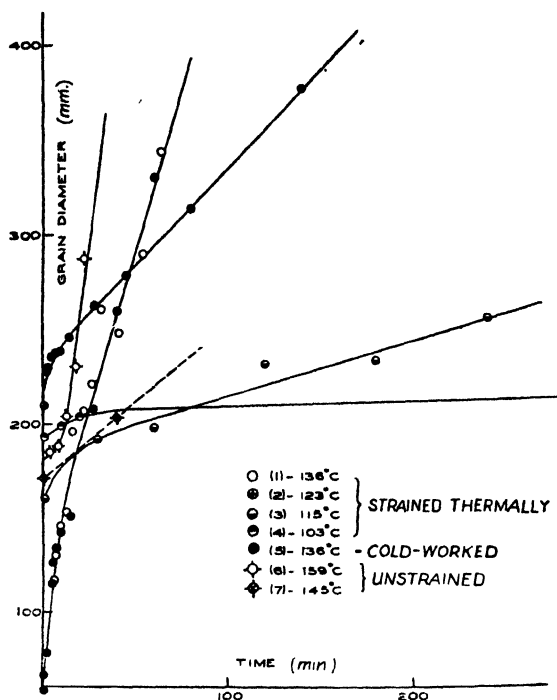


FIG. 4.—Grain growth curves for octachloropropane.

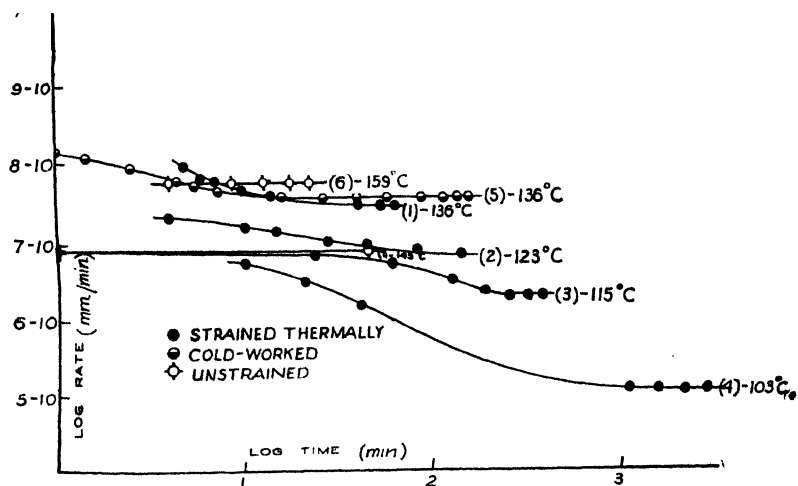


FIG. 5.—Rate-time curves for grain growth in octachloropropane.

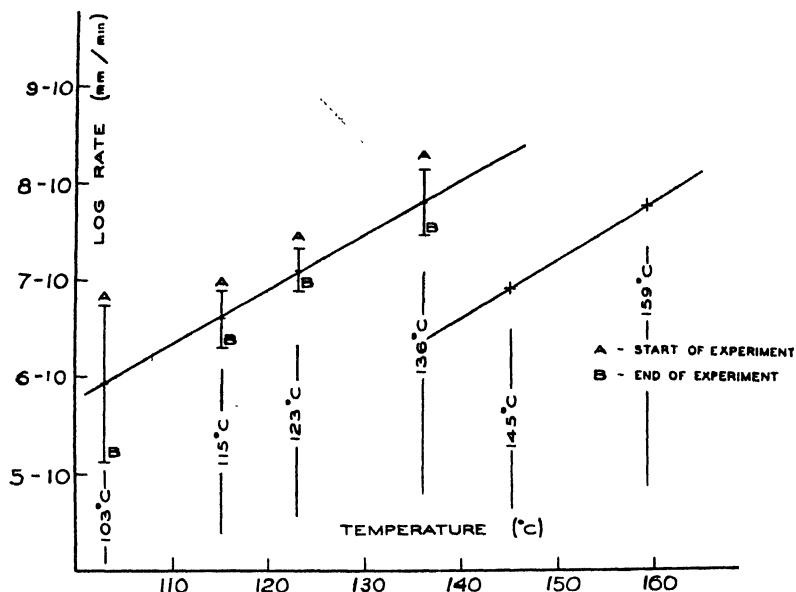


FIG. 6.—Rate-temperature curves for grain growth in octachloropropane.

### Discussion

Fig. 4 shows that the slope of the rate curve plotted against time is constant after an initial period and that the slope increases with increasing temperature. The equations for the linear portions are :

$$136^{\circ}\text{C} : D = 3.2M + 130 \quad . \quad . \quad . \quad . \quad (1)$$

$$123^{\circ}\text{C} : D = 1.1M + 230 \quad . \quad . \quad . \quad . \quad (2)$$

$$115^{\circ}\text{C} : D = 0.28M + 189 \quad . \quad . \quad . \quad . \quad (3)$$

$$103^{\circ}\text{C} : D = 0.029M + 205 \quad . \quad . \quad . \quad . \quad (4)$$

where  $D$  is the average grain diameter in microns and  $M$  is the time in minutes. The constant in each relation is, of course, fortuitous and depends only on the grain size of the original preparation.

These equations are equivalent to the expression given by Beck<sup>8</sup> :

$$D = K(tg + A)^n.$$

where  $K$  is the slope and  $A$  the imaginary time required for the grains to grow by boundary migration to an average size  $D$  at  $tg$ . In either case, however, the question is whether  $K$  is independent of  $A$  or, in the other case, whether  $S$  is independent of  $D$ , the intercept on the grain diameter ordinate. The fact that the slope is a linear function of temperature (shown below) as well as the fact that the  $D$  against time curves are also linear is strong evidence for the belief that  $\Delta D/\Delta M$  is independent of initial grain size.

A plot of the log slope against temperature is also very nearly linear and follows the relation,

$$\log S = 0.063 T + (2.10 - 10), \quad . \quad . \quad . \quad . \quad (5)$$

where  $S$  is the slope,  $\Delta D/\Delta T$ , and  $T$  is the temperature in  $^{\circ}\text{C}$ .

These relations show that boundary migration in metals is closely related to the same phenomenon in octachloropropane.

<sup>8</sup> Beck, *J. Appl. Physics*, 1948, **19**, 507.

Fig. 4 also shows that two different preparations, one strained thermally (Expt. 1) and a second strained mechanically (Expt. 5), show little difference in rate of increase of grain diameter as a function of time. This may have been coincidental in that the amount of strain induced by these two means may have been nearly equal.

The two Expt. 6 and 7 made on nearly unstrained crystals show that these two preparations grew at rates far below those predicted by eqn. (5) on the basis of Expt 1-5.

It is believed that the growth which occurred in Expt. 6 and 7 is partly the result of residual strain and partly of the tendency of the grains to form straight boundaries meeting at  $120^\circ$  angles. Since the interboundary angles for the preparations used in Expt. 6 and 7 are no nearer  $120^\circ$  than those used in Expt. 1-5, the decreased growth in Expt. 6 and 7 must be due to lack of lattice strain. In other words, lattice strain must be the most important factor causing boundary migration in octachloropropane.

TABLE II  
COMPARISON OF OBSERVED AND CALCULATED SLOPES

Expt.	Temperature	Slope	
		Observed	Calculated
7	$145^\circ \text{C}$	0.8	18
6	$159^\circ \text{C}$	7.2	126

Fig. 5 shows the smoothed rate data plotted in log form as a function of log time. These curves illustrate again that the rate is higher in the early stage of annealing and decreases quickly to a constant value. The constant rate is, of course, reached more rapidly the higher the temperature. These curves show again that Expt. 6 and 7, at  $159^\circ \text{C}$  and  $145^\circ \text{C}$  respectively, are lower than would be expected from an extrapolation of rates in Expt. 1-5. This figure shows the separate curves for Expt. 1 and 5 which were combined by smoothing in Fig. 4.

These data show that boundary migration in octachloropropane is very similar mathematically to boundary migration in metals. It is suggested that the mechanism by which boundary migration occurs in lattices of these two types is therefore similar and that boundary migration in metals can be studied to great advantage using the much simpler technique involved in studying octachloropropane.

As a result of the above work on octachloropropane it was decided to attempt to determine the effect of lattice strain on boundary migration in compounds of the DDT type. Unfortunately DDT itself could not be used since the crystal habit changes drastically with temperature of crystallization. However, TNT can be crystallized as broad rods over a wide temperature range. Accordingly an attempt was made to determine the effect of thermally induced lattice strain on boundary migration in TNT. First a small sample (5-10 mg.) of TNT was melted and cooled to about  $50^\circ \text{C}$  before crystallization. This preparation was then placed in an already heated hot-stage at  $78^\circ \text{C}$  and observed for a period of 40 min. During this time the crystals grew into adjacent crystals a distance of 0.5 mm. Fig. 1 shows two photomicrographs in this series, one taken at the end of 1 min., the second at the end of 4 min.

A second preparation of TNT was then melted and placed in the hot-stage at 80° C before crystallization occurred. On seeding, crystals of TNT were made to grow slowly into contact at right-angles. Observation of this and similar preparations over a period of 60 min. showed no sign of boundary migration. The conclusion from this information is that boundary migration in TNT and presumably in DDT and other compounds of this type is entirely due to lattice strain.

**Conclusion.**—Boundary migration in the octachloropropane and DDT types of crystal lattice is similar in the sense that lattice strain due either to cold working or temperature changes seems to be the principal motivating influence. The two differ, however, in two respects: (i) relative orientations of the neighbouring crystals are important for the DDT type and have little or no effect on compounds of the octachloropropane type; (ii) grain shape is important in controlling grain growth in compounds of the octachloropropane type and not important in compounds of the DDT type.

This dependence of boundary migration in crystals of the DDT type on relative orientation of the two crystals is more difficult to explain. This, however, has been resolved by the thought that all compounds of the octachloropropane type possess crystal lattices which are either cubic or, at least approximately plastically isotropic. On the other hand, crystals of the DDT type which show boundary migration are highly anisotropic compounds and must be elastically anisotropic. In other words, when crystals of this type, such as DDT, are subjected to pressure or to large temperature changes the resulting strain must be distributed anisotropically throughout the lattice and in such a way that the crystals grow most readily parallel to one definite direction, depending on the anisotropy of elasticity for that lattice.

This work was supported jointly by the Armour Research Foundation and the Research Corporation. Percy T. Cheng made some of these measurements. This help is gratefully acknowledged.

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## CRYSTAL GROWTH AT HIGH TEMPERATURES

BY S. ZERFOSS, L. R. JOHNSON AND P. H. EGLI

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Large single crystals can be grown from water solution, e.g., of K-alum or  $\text{NH}_4\text{H}_2\text{PO}_4$ , near room temperature because of ready solubility of the salts in water and their large temperature coefficient of solubility. For many other materials which possess low solubility in water or other solvents, some other technique involving higher temperatures must be used. The selection of the ideal growth technique depends naturally on the properties of the substance, particularly its melting point, the stability of its melt, and the possible occurrences of lower temperature inversions. Although single-crystal growth can be effected from melts of polynary composition, the most successful growth to date has been achieved with mono-mineralic melts. When a substance exhibits polymorphism, as quartz or nephelite, where the

desirable phases are not in equilibrium with the melt, the crystal must be grown from a binary or ternary melt, e.g., the ternary system  $\text{Na}_2\text{O}-\text{SiO}_2-\text{H}_2\text{O}$  in the case of quartz and the system  $\text{LiF}-\text{Na}_2\text{O}-\text{Al}_2\text{O}_3-\text{SiO}_2$  in the case of nephelite. Similar subterfuges are available for growth of other polymorphous substances; for example, the water-insoluble  $\text{HgI}_2$  (red form) can be grown successfully from a solution of  $\text{K}_2\text{HgI}_4$  saturated with  $\text{HgI}_2$  by lowering the temperature of the solution.

The growth of crystals from anhydrous melts or vapours can be accomplished in a variety of ways providing the substance melts congruently and has no lower temperature inversions:

(1) THE MOVING CRUCIBLE TECHNIQUE. A crucible containing the melt is slowly lowered through a fixed thermal gradient.<sup>1 2 3</sup>

(2) THE STATIONARY CRUCIBLE TECHNIQUE.<sup>4</sup> A crucible containing the melt is cooled in a slowly changing thermal gradient. This variation of (1) was used successfully by Stöber,<sup>5</sup> Stockbarger<sup>2</sup> and Phelps.<sup>6</sup> It does not appear to have any advantage over (1).

(3) THE KYROPOULOS TECHNIQUE.<sup>7</sup> A crystal seed is lowered into the melt and then slowly withdrawn through a gradient provided by cooling the seed. This technique has been used to grow crystals up to 30 cm. diam. ( $\text{NaCl}$ ). It might have special advantages for those crystals that show one strong directional-growth tendency.

(4) EUTECTIC MELT GROWTH TECHNIQUE.<sup>8 \*</sup> Through the use of fluxes a low-temperature phase may be induced to appear on the liquidus of the phase diagram and hence make growth of the phase possible, e.g., nephelite from a  $\text{LiF}-\text{NaAlSiO}_4$  melt, whereas growth from a mono-mineralic melt of the same phase would yield the undesired high-temperature modification, carnegieite. By cooling a melt, of which the desired substance is the primary phase, the resultant solid would consist of crystals of the primary phase in an eutectic matrix. Winkler<sup>8</sup> has discussed the relationship between the degree of supercooling and the size and number of the nephelite crystals. The difficulties of obtaining large single crystals by this technique appear enormous because of inability to prevent multiple nucleation.

(5) THE VERNEUIL TECHNIQUE.<sup>10</sup> Melting is accomplished by passing the powdered substance through one tube of an oxyhydrogen torch into the flame. The molten material is collected on a refractory rod and by suitable manipulation is converted into a single crystal.

<sup>1</sup> Bridgman, *Proc. Amer. Acad. Arts Sci.*, 1925, **60**, 305.

<sup>2</sup> Stockbarger, *OSRD Report*, No. 4690 (1944). *Rev. Sci. Instr.*, 1936, **7**, 133. *J. Opt. Soc. Amer.*, 1937, **27**, 416. *Rev. Sci. Instr.*, 1939, **10**, 205.

<sup>3</sup> Tuttle and Egli, *J. Chem. Physics*, 1946, **14**, 571.

<sup>4</sup> Strong, *Physic. Rev.*, 1930, **36**, 663.

<sup>5</sup> Stöber, *Z. Krist.*, 1923-4, **61**, 299.

<sup>6</sup> Phelps, *Chem. Eng. News*, 1948, **26**, 2453.

<sup>7</sup> Kyropoulos, *Z. anorg. Chem.*, 1926, **154**, 308. See also *B.I.O.S. Final Report* No. 468, No. 21, 22.

<sup>8</sup> Winkler, *Amer. Miner.*, 1947, **32**, 131; *Heidelberger Beiträge zur Miner. u. Petrographie*, 1947, **1**, 86.

<sup>9</sup> Matthias, *Physic. Rev.*, 1948, **73**, 808.

<sup>10</sup> Verneuil, *Compt. rend.*, 1902, **135**, 791. *Ann. Chim. Phys.*, 1904, **3**, 20.

\* Small single crystals of  $\text{BaTiO}_3$  have been grown \* by a similar operation (using an excess of  $\text{BaCl}_2$ , etc.) although the phase relationships are not known. Such "mineralization" is standard practice for the recrystallization of high-melting, slightly soluble materials.

(6) GROWTH FROM THE VAPOUR PHASE. (a) "Chemical reaction" in the gaseous state is carried out in a suitable thermal gradient, e.g., Cd vapour +  $H_2S$  yields CdS (greenockite) +  $H_2$ .<sup>11</sup>

(b) Volatilization of a pure substance and condensation in a gradient into single crystals, e.g., Se,  $I_2$ .<sup>12</sup> This technique is undeveloped but has been used to produce small crystals (up to  $5 \times 5 \times 10$  mm.) for a variety of substances. The difficulties of the technique lie in the impossibilities of setting a gradient of the proper character in a gas stream and providing a suitable heat sink for deposition of single-crystal material from a super-saturated gas. The Crystal Section has extended Frerichs' work in producing crystals of CdS having small amounts of luminescent-active additives.

TABLE I

Name	Composition	Size Boule Available * (in.)	Source **
Halide .. ..	NaCl	8	C
	NaBr	1	L (NRL)
Villiaumite .. ..	NaF	1½	L (NRL)
Sylvite .. ..	KCl	8	C
	(K,Rb) Cl	½	L (NRL)
	KBr	8	C
	KI	1½	L (NRL)
	LiF	8	C
Nantockite .. ..	CuCl	½	L (NRL)
Cerargyrite .. ..	AgCl	5	C
Bromyrite .. ..	AgBr	3	L (NRL)
	TiCl	3	L (ERDL) (NRL)
	TiBr	3	L (ERDL) (NRL)
KRS 6 .. ..	Tl (Cl,Br)	3	L (ERDL) (NRL)
KRS 5 .. ..	Tl (Br,I)	5	L (ERDL) (NRL)
Fluorite .. ..	CaF <sub>2</sub>	6	L
	BaF <sub>2</sub>	4	L
	MnF <sub>2</sub>	?	L
	PbCl <sub>2</sub>	½	L (NRL)
	PbBr <sub>2</sub>	½	L (NRL)
	PbI <sub>2</sub>	1½	L (NRL)
	CdI <sub>2</sub>	1½	L (NRL)
Scheelite .. ..	CaWO <sub>4</sub>	½	CL (NRL)
	ZnWO <sub>4</sub>	½	CL (NRL)
	CdWO <sub>4</sub>	½	L (NRL)
	NaNO <sub>3</sub>	½	L (NRL)
	NaNO <sub>3</sub>	3	L
	AsI <sub>3</sub>	½	L (NRL)

\* Size available—diameter of cylindrical boules of 2 in.—4 in. length.

\*\* Abbreviations: C — Commercially produced.

L — Academic laboratories.

(NRL) — Crystal Section, Naval Research Laboratory.

(ERDL) — Engineer Research and Development Laboratory, Ft. Belvoir.

The Crystal Section of the Naval Research Laboratory has been using techniques ((1), moving crucible), ((5), Verneuil) and ((6), vapour growth) to produce a variety of small single crystals for research purposes (see Table I). In this paper an attempt will be made to correlate the various factors influencing growth. Table I is a summary list of crystals grown by various industrial and academic laboratories based on information available to us. Of the six techniques, only (1), (2) and (5) are in current industrial use for

<sup>11</sup> Frerichs, *Physic. Rev.*, 1947, **72**, 594.

<sup>12</sup> Vasko, *Sklarske rozhledy*, 1946, **6-7**, 98.

the mass production of single crystals. The other techniques have been little used because of inherent difficulties in control of single-crystal growth but are potential techniques for unexplored materials.

If none of the melt techniques is suitable because of the existence of lower temperature inversions in a substance, there is a method of last resort—that of growth under hydrothermal conditions—in the presence of water at elevated temperatures and pressures in a closed system. Despite theoretical and experimental difficulties much attention is being paid to this technique but it is outside the scope of this report.

### Experimental

**Technique No. 1.** By far the most popular technique is that of moving a crucible through a fixed gradient.\* It is capable of producing much larger crystals because of the ease of gradient control. A cylindrical crucible with a cone-shaped tip containing the melt is slowly lowered from some temperature above the liquidus temperature down through the fixed gradient of the furnace.

Presumably, when the tip of the crucible reaches the liquidus temperature, the small volume of the tip favours the incidence and development of one nucleus at the expense of all others and thereafter conditions are so maintained that this initial crystal will grow vertically and assume the shape of the crucible. For this purpose ideal conditions obtain when the isotherms are horizontal. Their distribution of spacing will characterize the gradient. Naturally, the control of the gradient, speed of lowering and the purity and composition of the crystal are important factors. If adequate controls are not imposed, several nuclei will be competing for the material from the freezing liquid thereby yielding a polycrystalline boule.

**THE CONDITIONS OF CRYSTALLIZATION.** The conditions for producing a single crystal, though dominated by the purity of the charge, depend upon the rate at which heat is withdrawn from the melt. The heat sink is provided in two ways—the thermal gradient and the speed at which the crucible passes through the gradient. Too small a gradient or too rapid lowering of the crucible favour the growth of many nuclei leading to polycrystalline boules. The gradients available for growth are limited by furnace design and by the temperature of operation. Numerous unsuccessful attempts have been made to systematize these data and reduce them to an equation. The difficulties lie in the inability to specify the exact conditions for obtaining single crystallinity.

The furnace used in technique No. 1 has been described.<sup>2</sup> It consists of an upper and lower section separately wound and controlled. The gradient along the furnace length can be modified by adjusting the heat input into the separate sections. By supplying heat to the upper section only, one obtains a temperature distribution with the high point near the bottom of the upper section and a sharp gradient. This gradient can be reduced by supplying heat to the lower section. It can be intensified by the use of a baffle between the two furnace sections. The baffle is a thin, flat annulus of metal or ceramic material, the inner diameter of which is large enough to permit passage of the crucible. Typical data on the effect of the baffle are given as follows:

NRL furnace 6-1948, 28 in. high, inner tube diameter = 4 in.

Temp. at 14 in. level = 464° C.

Thermal gradient at 14 in. level with baffle = 74°/in.

without baffle = 44°/in.

The question of whether or not the baffle has any particular efficacy in favouring single-crystal growth over the plain furnace has not been settled since it involves the question of the optimum gradient. Any of the crystals mentioned can be grown without a baffle, and we can report no detectable improvement from the use of a baffle.

\* This gradient is somewhat modified by the change of position of the crucible during lowering but this factor is probably small, provided the heat capacity of the crystal is small compared to that of the whole system.



It is apparent from our experience that the diameter of the muffle does not play a role in the gradient providing it exceeds the crucible diameter by a factor of 1.5. The isotherms within the crucible for this condition are flat as shown by the freezing surface of the single-crystal part of several incompletely crystallized boules. This temperature control can be accomplished by any of a variety of constant-temperature regulators,<sup>13</sup> but we have found the use of a constant-voltage transformer (saturable-reactor type) sufficient to maintain the temperature  $\pm 1.0^\circ \text{C}$  in the range  $300^\circ\text{--}1000^\circ \text{C}$  for crucibles up to  $1\frac{1}{2}$  in. diam. For larger crucibles more elaborate controls are required ( $\pm 0.2^\circ \text{C}$ ).

Typical gradients used for successful growth in our laboratory are as follows :

Melting Point $^\circ \text{C}$	Gradient $^\circ \text{C/in.}$	Expt. No.
AgCl	55	NRL 20-6
TiCl	45	NRL 22-6
KI	64	NRL 114-7
NaF	45	NRL 63-1

In our experience gradients larger than  $75^\circ \text{C/in.}$  can be used if available. The lower limit for successful growth of medium-sized crystals is  $35^\circ \text{C/in.}$  The higher temperature runs operate under higher gradients because of normal furnace design.

The lowering of the crucible is accomplished by a gear assemblage attached to the support rod and operated by a constant-speed motor. The effect of the speed of lowering is related to the gradient since both perform the same heat-sink operation on the melt but the relationship is not a simple one and has not been worked out. For medium-sized crucibles (up to  $1\frac{1}{2}$  in. diam.) a speed of  $0.12$  in./hr. or  $0.32$  cm./hr. is optimum. Slower speeds are not detrimental providing temperature control is adequate.

The stability of the furnace assemblage, i.e., the amount of vibration suffered by the crystal during growth, is apparently not significant. In fact, some vibration may be essential to the prevention of extreme supercooling with subsequent multiple crystallization. To test the effect of excessive vibration, crystals of AgCl were grown with a buzzer attached loosely to the support rod. Satisfactory single crystals resulted in two runs.

The shape of the crucible commonly used by the various investigators is a cylinder with a  $60^\circ\text{--}135^\circ$  conical tip. However, there appears to be nothing critical about the angle of the conical tip. Successful single crystals  $\frac{3}{4}$  in. to  $1\frac{1}{2}$  in. diam. of AgCl, TiCl, and TiBr were grown in flat-bottomed crucibles at lowering speeds of  $0.12$  in./hr. in a gradient of  $45^\circ \text{C/in.}$

The choice of crucible material depends on the nature of the crystal to be grown, its chemical reaction with the crucible, and the temperature of operation. The following crucible materials have been used in our laboratory or reported in the literature.

#### TYPICAL CRUCIBLE MATERIALS

CRYSTAL	CRUCIBLE MATERIAL	REMARKS
Alkali halides	Platinum *	Some adherence
Lead halides	Silica glass	Sealed crucible
Thallous halides	Pyrex glass	Little adherence
Silver halides	Pyrex glass	Some adherence **
Divalent tungstates	Platinum ***	
Fluorite	Carbon	

\* Strong used an iron crucible for alkali halides (in an  $\text{H}_2$  atmosphere).<sup>4</sup>

\*\* It is known that AgCl or other silver salts react with alkali glasses exchanging Ag for Na ions at the surface. AgCl adheres strongly to such altered glasses.

\*\*\* Considerable difficulty was experienced in preparing leak-free crucibles for these temperatures.

<sup>13</sup> *Temperature, Its Measurement and Control in Science and Industry*, Amer. Inst. Physics (Reinhold, N.Y., 1941).

As far as temperature stability goes, Pyrex glass is suitable up to  $500^{\circ}\text{C}$ , silica glass can be used up to  $1100^{\circ}\text{C}$  and platinum is suitable up to its melting point. Most single crystals can be grown in open crucibles in air. Some exceptions are easily oxidized substances like the lead halides. Fluorite is reported to hydrolyze in air during growth.

In the case of the glass crucibles the support rod is an extension of the tip of the crucible. The metal crucibles are supported on metal cone supports. The removal of the crystal from the crucible has been done by inverting the crucible immediately after crystallization is complete and raising the temperature rapidly above the liquidus whereupon a thin layer of the crystal melts and the crystal drops out of the crucible. For medium-sized crucibles with proper annealing of the crystal, this is not necessary. The crystal can be removed by cracking the crucible; in fact, the crucible is usually fractured during annealing.

**THE CHARGE.** The factor of purity of the initial charge is a complex and important one since it probably dominates all other factors in determining the final single crystallinity of the boule. An impurity in the charge is foreign both to the composition of the melt and to the lattice of the crystal. It is usually insoluble in the crystal and hence does not favour single-crystal growth but leads to multiple crystals and cloudy boules. For example,  $\text{TiCl}_3$  is completely soluble in  $\text{TiBr}_3$  in the melt and crystal, and good single crystals of solid solutions can be had. However, the presence of more than  $0.1\%$   $\text{TiCl}_3$  in  $\text{AgCl}$  (in which it is sparingly soluble) yields cloudy polycrystalline boules. Hence, in the latter case it is a definite impurity. In fact, a melt containing  $0.5\%$   $\text{TiCl}_3$  in  $\text{AgCl}$  can be converted to a poor single crystal containing less than  $0.1\%$ , the remainder having been rejected during growth.

Generally speaking, the usual c.p. chemicals are not suitable. For each chemical a purification scheme must be worked out to provide sufficient purity for the growth technique, and in some cases additional purification must be carried out to satisfy more rigid specifications for a particular material. However, it is slowly being recognized, for crystals grown from the melt or from water solution, that *extreme* purity is not always desirable and often leads to poor growth.

The degree of perfection of many crystals can be improved through the use of small amounts of additives, e.g.,  $\text{Pb}^{++}$  in  $\text{NaCl}$  or  $\text{Ti}^{+}$  in  $\text{KI}$ . No correlation can be made between the chemical nature of the additive and its effectiveness in improving crystal quality beyond the statement that most effective additives are large, easily polarizable cations. The difficulties in obtaining a single-crystal boule of a variety of materials vary with the chemical nature of the material, in a manner as yet not determined. For example, rather extreme controls are required, on matters of purity, etc., for such materials as  $\text{PbCl}_2$  and  $\text{CaWO}_4$ , while on the other extreme  $\text{AgCl}$  can be converted into single-crystal material under almost any conditions.

On the basis of general phase-rule considerations, the crystallization of a solid solution by the melt technique should yield a fractionated boule with the tip material richer in the higher melting component. For normal, solid-solution behaviour one might predict that a homogeneous, solid-solution single crystal could not be grown. For example, a crystal ( $2\text{ in.} \times \frac{3}{4}\text{ in. diam.}$ ) grown from a melt of  $\text{AgCl}$  and  $\text{NaCl}$  (NRL-13-X) the top of the boule analyzed  $76\text{ mol-}\%$   $\text{AgCl}$  while the tip of the boule analyzed  $72\%$   $\text{AgCl}$ . A similar situation obtained during the growth of solid solutions in the system  $\text{KCl-KBr}$  (in this latter the fractionation was detected by refractive index measurements rather than chemical analysis). The solid solution  $42\text{ mol-}\%$   $\text{TiBr}_3$ - $58\text{ mol-}\%$   $\text{TiI}_3$  may show some fractionation during growth. McFee<sup>14</sup> has given quantitative data on the self-purification of impure crystals of  $\text{NaCl}$  during growth.

**Technique No. 5.** The available crucible materials limit the crucible-melt technique to temperatures below  $1700^{\circ}\text{C}$ . Crucibles are available for higher temperatures but they are readily attacked by the various melts and hence usually unsuitable.

In 1891 a Swiss worker, Verneuil, developed a method for growth of crystals

<sup>14</sup> McFee, *J. Chem. Physics*, 1947, **15**, 856.

of highly refractory materials by fusing the material in the flame of an oxygen-hydrogen torch.<sup>16</sup> In this process, fine powder is introduced into the oxygen tube of the burner. As the powder enters the flame it is melted and is collected on a refractory rod. By suitable manipulation this melted material can be caused to grow into a single crystal. Initially the powder is allowed to accumulate as a cone of semi-melted material on the support and then the tip of this cone is melted by adjusting the gases, and the flow of powder is increased. If this initial melting operation is carried out under proper control, a single crystal results which may be extended by smooth addition of more material as the rod is lowered. Some operators use oriented seeds attached to the rod as the original material to which additional material is added through the flame.

The published literature on the details of this process is quite scanty. The successful growth operation depends upon the *skill* of the operator in manipulating the flame and feed and in initiating the single-crystal tip. Further growth proceeds continuously as the feed material melts and is assimilated into the growing crystal. The purity of the starting material must be quite high. The same concepts apply to the question of purity in this material as in the melt-crucible growth. For example, in the growth of sapphire boules the  $\text{Al}_2\text{O}_3$  is prepared by calcining a twice-recrystallized ammonium alum. The growing crystal purifies itself by "scumming-off" impurities to the liquid surface of the boule. If the feed material is too impure, this scum covers the surface, and growth is interrupted.<sup>15</sup>

In the past, the crystals grown by this technique have been limited to corundum (sapphire), corundum coloured by small amounts of other oxides (ruby, alexandrite, etc.) and a spinel solid solution ( $\text{MgAl}_2\text{O}_4 + \text{excess Al}_2\text{O}_3$ ). In the past year, several new crystals have been added of which we can list:

(1) **RUTILE.**<sup>16</sup> This crystal, grown up to 75 carats, emerges from the furnace as a black, oxygen-deficient boule which can be bleached in  $\text{O}_2$  into a clear, light-yellow material with rather unusual optical and electrical properties.

(2) **SCHEELITE**— $\text{CaWO}_4$ .<sup>17 18</sup> This material (of interest as the light emitter in scintillation counters) has been grown up to  $\frac{1}{4}$  in. diam. with little difficulty.

*Crystal Section,  
Naval Research Laboratory,  
Washington, D.C.*

<sup>15</sup> Moore, Jr., private communication, 1948.

<sup>16</sup> Titanium Division, National Lead Co., *Sci. Newsletter*, Oct. 1947.

<sup>17</sup> Linde Air Products Corp., *Chem. Eng. News*, 1949, **27**, 48.

<sup>18</sup> Zerfoss, Johnson and Imber, *Physic. Rev.*, 1949, **75**, 320.

## SOME ASPECTS OF THE GROWTH OF QUARTZ CRYSTALS \*

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The aspects of laboratory quartz research discussed in this paper are three-fold. (1) Quantitative growth results and observations related to growth experiments are presented. Growth was obtained in dilute alkali halide

\* The results and interpretations presented here are derived from work supported on contract between Antioch College and the U.S. Army Signal Corps through its Signal Corps Engineering Laboratories at Fort Monmouth, New Jersey. The senior author is Professor of Geology at Antioch College; from 1943 to 1946 he was associated with the U.S. Army Signal Corps in the research and development activities related to quartz oscillator units. In late 1945 he participated in the interrogation of German scientists, among them Rickard Nacken, who had worked in the search for alternatives to natural quartz. Dr. Owen is Professor of Physics and Dr. Corwin is Associate Professor of Chemistry, both at Antioch College.

solutions at 400° C with a nominal zero thermal gradient, utilizing vitreous silica as source material and crystalline seed plates. (2) In supplementary experiments designed to investigate the nature of the solutions in the critical state apparatus was developed to record automatically the impedances of the liquid and vapour phases as they approach and pass into the supercritical state. The current results of these investigations are described since the impedance technique gives promise of having both practical and theoretical value. (3) Finally, certain tentative, theoretical interpretations regarding the nature of the  $\text{SiO}_2\text{-H}_2\text{O}$ -metal ion relationships, based in part on X-ray analysis, are outlined. This paper is in the nature of a progress report of work which is continuing. It represents data and conclusions as of approximately 1st February, 1949.

### Growth in Alkali Halide Solutions

The selection of the alkali halide solutions as the solvents in laboratory quartz experiments resulted from a survey of the analyses of the liquid inclusions in natural quartz. Na and Cl were found to be the commonest components reported. Early results in this project gave growth when fused silica, rather than quartz, was used as source material; fused silica has been consistently, although not exclusively, used for this purpose. Seed plates are natural quartz, either AT-cut oscillator blanks or plates cut parallel to the minor rhombohedral face, approximately  $0.5 \times 0.6 \times 0.030$  in. in size and weighing close to 0.400 g.

The autoclave equipment consists of several 250 ml. bombs made by the Parr Instrument Company from stainless steels 347 and 316. Some difficulty has been experienced in obtaining steel free from defects. The interior of the bomb is a cylindrical cavity  $3\frac{1}{2}$  in. deep by  $2\frac{1}{2}$  in. in diameter. The centre of the lid has a small eyelet for suspending the seed plate. Just off centre a thermowell tube extends  $\frac{3}{4}$  in. into the cavity. Accessory equipment includes a junction block above the lid with a blow-out safety disc, a needle valve outlet and a tubing connection to a 0-10,000 psi Bourdon-type gauge. The closure gasket is copper.

The heater is a vertical insulated cylinder with two Calrod units coiled one above the other; each is controlled by its own variable transformer. Thus the heat is supplied through the walls of the autoclave rather than at the bottom. Temperature control is supplied by Leeds and Northrup proportioning controllers through mechanical relays. Temperature observations are recorded automatically from two thermocouples, one placed in the thermowell in the lid and the second inserted in a copper block insulated from the furnace wall but supporting the bottom of the autoclave. The thermal gradient in the autoclave is controlled in two ways: (1) the input to the lower and upper heating coils may be varied by adjusting the variable transformers; (2) the radiation from the upper surface can be varied by piling on, or removing, loose insulation.

An operating temperature of 400° C was selected for the reasons that it was sufficiently above the critical temperature to ensure the existence of the supercritical state within the autoclaves and for all degrees of filling under 65 % was within the safe limits of the equipment. The thermal gradient, although controlled closely, is probably not less than 1° C, or more than 5° C, warmer at the bottom than at the top.

Several concentrations of sodium chloride and several degrees of filling or "charge" were systematically explored in series. In the first series the solutions were "neutral," i.e., the NaCl was dissolved in distilled water of pH ranging around 6. At least two experiments were done for each set of conditions. The single figures in Table I represent the averages of

consistent results. Forty-eight hours at temperature was the standard nominal period.

TABLE I  
AVERAGE % INCREASE IN WEIGHT OF SEED PLATE.  
SODIUM CHLORIDE PH 6-7. 48 HR. AT 400° C. 0°  
THERMAL GRADIENT. AVERAGE WEIGHT OF SEED  
PLATE 0.400 G.

% Charge	N/40	N/30	N/20	N/10	N/2	N
50	2	3.5	2.5	2.5	2	-40
40	1.5	2	1.5	2	-3	-13
30	3	3	2	4.5	3	4
20		5		10		7

A second series of experiments was undertaken in which the initial pH of the solution was adjusted to  $10 \pm 0.5$  by the addition of a few drops of concentrated NaOH. A slightly different range of concentrations was used for the second series. Otherwise the conditions of experimentation shown in Table II are the same as those shown in Table I. The contrast in results between the two Tables indicates the effectiveness of the increased alkalinity. In every case the terminal pH had a value in the range of 7 to 5.

TABLE II  
AVERAGE % INCREASE IN WEIGHT OF SEED PLATE.  
NaCl ADJUSTED TO PH 10 WITH NaOH 48 HR.  
AT 400° C. 0° THERMAL GRADIENT. AVERAGE  
WEIGHT OF SEED PLATE 0.400 G.

% Charge	N/40	N/10	N/2	3N/4	N
50	120	4	4	-76	-10
40	5	5	3.5	-36	-13
30	4	10	8	0	6
20	7	12	25	10	10

Following the discovery of the high-yield region at N/40 and 50 % charge, exploration of concentrations down to N/200 and as high as 60 % showed less growth than with N/40, 50 %.

Experiments using KCl, KBr and NaBr, N/40 and 50 % charge, yielded growth. Only the NaBr experiments indicated results comparable to NaCl.

TABLE III  
GROWTH IN SOLUTIONS OTHER THAN  
NaCl N/40, 50 % CHARGE. OTHER  
CONDITIONS AS IN TABLE II

Solution	Growth %
KCl	66
NaBr	103
KBr	58

TABLE IV  
EFFECT OF AMOUNT OF FUSED SILICA  
SOURCE MATERIAL (VITREOSIL). OTHER  
CONDITIONS AS IN TABLE II

Grams of Source	Growth %
0.65	52.4
1.35	80.7
1.80	111.0
2.60	129.0
2.65	132.0
15.1	140.0
16.1	109.0

The relation of amount of source material (fused silica) to growth on a seed plate of given size shows a saturation phenomenon. Small amounts of

source yield small growth. But source material in excess of 2.6 g. shows little or no more growth on the seed plate than when 2.6 g. of fused silica is used as a source.

The source residue of the first run (0.65 g.) showed in X-ray analysis to be entirely quartz, whereas the residues from the last two (15.1 and 16.1 g.) showed some cristobalite present with quartz.

The relation of amount of growth to length of time represents a confused picture. Experiments continued in excess of 48 hr. tend to give less growth than those of the two-day period.

TABLE V

LENGTH OF TIME. CONC. N/40, 50 % CHARGE, pH 10, 0° THERMAL GRADIENT 0.400 G. SEED PLATES

Solution	Time (hr.)	Growth %	Remarks
NaCl	48	120	Average of several runs
NaCl	72	25	Average of several runs
KCl	24	63.5	One run with leakage
KCl	48	66	Average of two runs
KCl	96	73.9	Average of two runs
KBr	48	58.2	Average of two runs
KBr	96	58.4	Average of two runs

A striking characteristic of the process is the contrast between the initial alkalinity, adjusted in several series of experiments to a nominal pH 10, and the terminal pH which invariably shows less alkalinity and may even be on the acid side. The factors causing the variability in the terminal pH have not been identified with certainty. It does not bear a direct relationship either to time or to amount of growth. Devitrification of the fused silica source is pronounced and is another characteristic of the process. The fused material becomes either granular quartz or a mixture of quartz and cristobalite. It has not been possible to observe if the change in alkalinity is related to the devitrification.

Attempts have been made to counteract both changes. To maintain alkalinity, buffered solutions have been tried. Injection of alkali and manual regeneration of the solutions have been attempted. None of these has given substantial success as yet. The devitrification of fused silica led to the experimentation with other solutions and with other source materials. This phase of the investigation is still in progress.

In summary it should be pointed out that the method pursued here is dependent on the difference in solubilities of the solid forms of silica, particularly fused silica, and quartz. It is distinctly different from the techniques which utilize large thermal gradients and circulation from an under-saturated source zone to a supersaturated locus of crystallization. The phase-solubility transfer method yields rapid growth of good quality but is limited by a time factor which is associated with decreasing alkalinity and devitrification of fused silica source material.

### Impedance Studies

The introduction of an insulated electrical lead into the internal cavity of a stainless steel bomb has made it possible to observe certain changes in the liquid and vapour phases with temperature. The electrical lead is an airplane-type spark plug furnished by the AC Spark Plug Division of General Motors Corp. It consists of an electrode centred in a core of fused  $\text{Al}_2\text{O}_3$ . The core in turn is gasketed with copper in a threaded metal jacket which screws into the bomb. Another copper gasket is used to seal the

plug to the bomb. The outer end of the electrode is provided with a screw connector for one wire. The circuit is completed by a second wire attached to the wall of the bomb.

Best results have been obtained in a special 5 in. long 18 ml. tubular stainless steel bomb. One end is solid; a spark plug serves as the closure for the other end. The bomb is heated in a furnace which can be inverted so that readings may be taken alternately in the liquid and vapour phases. This method is preferred at present to the use of a two-plug bomb because the incidence of leakage is less with one plug than with two, and because there is no question of variation in impedance due to the presence of two plugs.

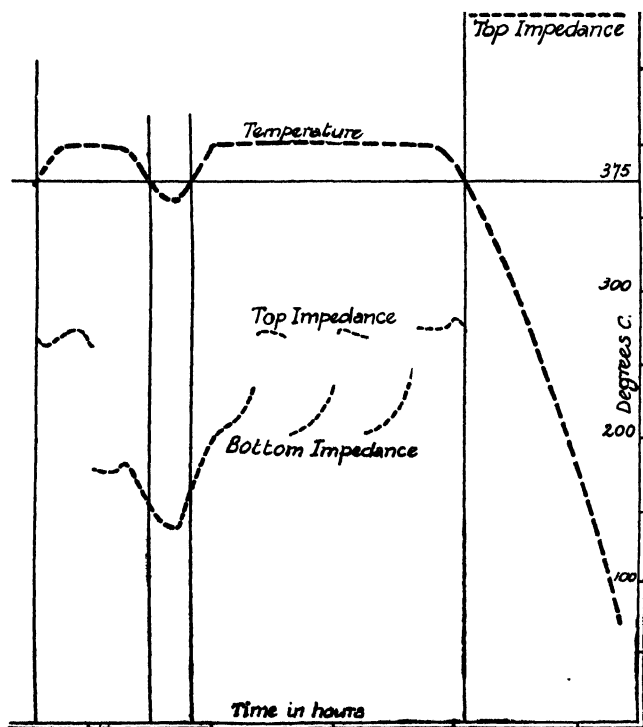


FIG. 1.—N/40 NaCl 40 %. Scale 100 ohms.

A voltage regulated signal generator supplies a 1 KC current to the spark plug circuit. The impedance is compared with a known value in a branch circuit. This voltage is rectified and, after suitable amplification, is applied to a potentiometer-type recorder of the kind ordinarily used for thermocouple temperature records. The ordinary temperature scale on the paper chart can be calibrated by substituting known resistances in place of the bomb. The instrumental and recording arrangement yields printed records of impedance and bomb-wall temperature on the same time chart.

Investigations are in progress to discover the basic patterns of various concentrations of NaCl and other alkali halides, with and without the alkalinity adjusted, with and without silica present, in several degrees of filling. Enough consistent data have been secured to permit certain comments and conclusions.

(1) The technique is successful in showing changes in the impedance-related properties of both the liquid and gas phases of the solutions so far used, as they approach and enter the supercritical state.

(2) The records of the top phase of the bomb clearly distinguish between different degrees of filling. The variations in bottom readings are small with differences in filling.

(3) So far variations in alkalinity have not been identified.

(4) The presence of silica has shown distinctive but anomalous behaviour, not subject to repetition in detail, but predictable as to general character. In particular, the impedance of the top phase seems to be lowered by the presence of silica.

The nature of the patterns obtained is shown in Fig. 1 and 2. Fig. 1 is the tracing of a chart of temperature and impedance for a 40 % charge of

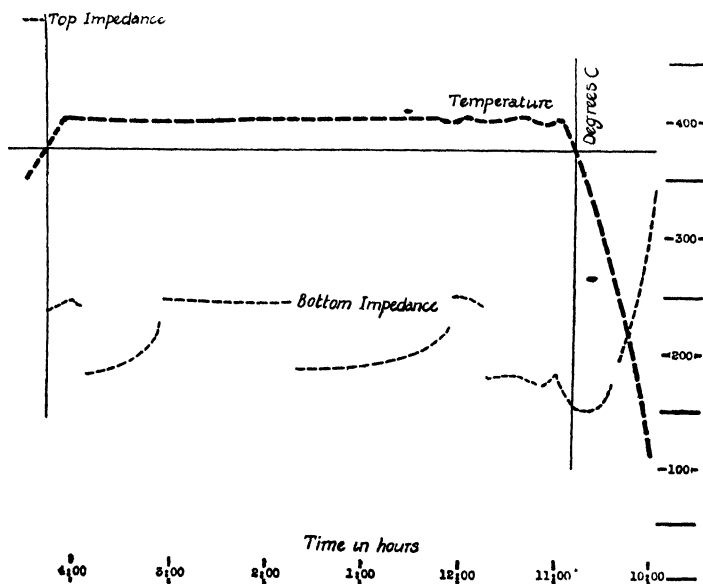


FIG. 2.—N/40 NaCl 40 %. Scale 100 ohms.

N/40 NaCl. The record begins at the right with a rapidly rising temperature curve. The high impedance of the top electrode (vapour phase) appears at the upper right. The nature of the apparatus is such that the scale as used for this record places 100 ohms near the top; very high values are not distinguishable. Below 100 ohms the scale is reasonably linear. When the temperature reaches  $375^{\circ} \pm 1^{\circ}$  the top impedance drops abruptly and levels off at 55 ohms as the temperature levels off at  $400^{\circ}\text{C}$ . The break in the record indicates that the furnace and bomb were inverted. The bottom impedance drops abruptly and then curves downwards, apparently approaching a firm value. The reading which the first (right-hand) down curve thus approaches asymptotically is 35 ohms. When the furnace and bomb are reinverted, so that the electrode measures top impedance, the break is sharp, the impedance returning to its previous value.

Fig. 2 shows the same conditions except that the record begins with the electrode at the bottom (liquid phase). The times when the furnace and bomb were inverted are apparent from the impedance shifts. The curves show



the response of impedance to temperature changes particularly at the bottom position. It also shows the abrupt break in top impedance when the cooling temperature curve passes through 375° C. The remarkable contrast in the variation of top impedance with charge and the absence of variation of bottom impedance with charge is shown in Table VI.

TABLE VI  
IMPEDANCE (Z) VARIATION WITH BOMB CHARGE  
N/40 NaCl AT 400° C. VOLUME OF BOMB 18 ML.

Charge (ml.)	Bottom Z (ohms)	Top Z (ohms)
8	35	55
6.4	35	80
6.0	—*	120
5.4	38	235
5.0	36	330
4.0	—*	1000

\* In these records the downward curve was not permitted to continue to the point where a satisfactory reading could subsequently be made; in both cases the number can be said certainly to be less than 50 and probably less than 40.

The consistency of readings is shown by Table VII in which are tabulated the impedance readings for several 40 % charges of N/40 NaCl.

TABLE VII  
IMPEDANCE CHARACTERISTICS OF 40 % CHARGE, N/40 NaCl

Minimum Z (ohms)	Temp. Min. Z	Bottom Z 400° C	Top Z 400° C
25	308° C	33	54
25	320	33	51
29	340	35	50
27	336	35	58
28	312	38	57

Measurements made with bombs from which air has been evacuated give curves which are very similar to those already described. Since the bombs were not designed to be evacuated the numerical results obtained are not precise enough for direct comparison. The same is true of experiments made with water with no sodium chloride, from which the air has been removed. The difference between top and bottom impedances remains and the general forms of the curves are similar.

Space does not permit discussion of the implications of the data in regard to the nature of the critical state. The possible applications to the observations and control of quartz growth appear promising. But much more work must be done before that step can be taken.

### Chemical Theory

In addition to the several series of growth experiments and the impedance studies, investigations have been made for the express purpose of establishing a hypothetical interpretation of the growth process. These include: (1) several 250 ml. autoclave runs using buffered solutions either as the growth medium or as the alkalizing agent; (2) 250 ml. autoclave trials of several different source materials; (3) X-ray examinations of the residual source materials and of the residual solutions after evaporation to dryness.

A summary of the pertinent facts follows: (1) the maximum growth occurs with fused silica in 48 hr. or less followed by cessation of growth and in some cases by resolution; (2) a decrease in the alkalinity shows a depletion in the available  $\text{OH}^-$  ion even when buffered solutions are used; (3) fused silica as source material gives excellent growth but devitrifies, becoming either quartz or a mixture of quartz and cristobalite; (4) quartz used as source does not transfer quartz to the seed plate, chalcedony promotes very slight growth, natural cristobalite yields a moderate increase in seed plate weight; (5) quartz, chalcedony and cristobalite remain unchanged, i.e., do not show modification like the change of fused silica to quartz and cristobalite; (6) the solution residuals when analyzed by X-ray indicate the presence of NaCl (in the NaCl experiments) together with non-crystalline silica.

The conclusions, which must be regarded as tentative, are not readily summarized and require more elaboration and explanation than space permits. In general it can be said that:

(1) The growth which occurs when fused silica and cristobalite are used as source material, together with the small or negative results with quartz and chalcedony, emphasizes the conclusion that the process is essentially based on the solubility differences of the several forms of silica. This statement is consistent with the generally accepted values of the vapour pressure of the several forms. Both solubilities and vapour pressures reflect the internal energies of the several forms.

(2) The solution of silica in dilute NaCl, pH 10 (NaOH) is probably not simple solution in the sense of the dispersal of  $\text{SiO}_2$  ions in the solvent. Disintegrative reaction is involved, more likely a series of such reactions, in which Na silicates are formed. Silicon tetrafluoride is a gas at ordinary temperatures. Arguing from the similarity in size and weight of OH and F, it seems possible that  $\text{Si}(\text{OH})_4$ , particularly at high temperature, may be a gas; likewise for  $\text{SiO}_4$  ions. These solution-reaction products are assumed to be less stable in the presence of quartz than in the presence of fused silica. Growth on the seed plate should return  $\text{OH}^-$  ions to the solution, maintaining the alkalinity. The process of growth, moreover, should leave some residual silicates in the solution. This residual does not appear in the X-ray data as crystalline silicates but as amorphous silica.

(3) The solution of the fused silica source is considered as occurring by the replacement one at a time of the oxygen atoms on the apexes of the silica tetrahedra by OH and ONa groups, thus breaking the bonds to the adjacent tetrahedra. During this process the alkalinity is depleted because of distribution of OH groups over the increasing surfaces. It is possible that this process also brings about the devitrification of the fused silica by giving opportunity for any vague structural nuclei of cristobalite and quartz in the fused silica to orient and complete their structures.

Although the tentative nature of these conclusions should be repeatedly emphasized, several lines of investigation are clearly suggested. (1) Devitrification of fused silica must be regulated or inhibited either by solutions or other operating conditions which discourage it. This answer is probably not easy. As an alternative to a direct answer, suspended fused silica might be injected into the autoclave to renew the source supply. (2) Source material which does not suffer modification can be sought. Cristobalite gives some promise and is currently under investigation. (3) The alkaline level may need to be maintained by injection to keep the transfer active.

### General Conclusions

The process of quartz growth by differential phase solubility gives rapid growth of excellent quality at temperatures above the critical point. Its

limitation is the cessation of growth after a short period. The stoppage is related apparently to a series of complex silicate solution reactions which result in diminished alkalinity and devitrification of vitreous source material. The impedance studies show promise as a means of developing a technique for observing and controlling the progress of the reactions. Since the quality of product is good and the process is rapid, investigations are continuing in an effort to overcome the limitations.

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## THE ROLE OF DIFFUSION POTENTIALS IN THE GROWTH OF IONIC CRYSTALS

BY A. R. UBBELOHDE

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The object of this paper is to draw attention to certain electrical effects which appear to be significant for the growth of crystals. Experimental study of these effects is still in too early a stage to permit a fully connected account of all the phenomena. Nevertheless some discussion of the role of electrical effects in the growth of ionic and polar crystals is desirable in reviewing the field of crystal growth as a whole.

### Diffusion Potentials in the Neighbourhood of Ionic Crystals.

—The different mobilities in solution of the positive and negative ions which go to form an ionic crystal must normally lead to the establishment of diffusion potentials in the neighbourhood of the crystals. Such diffusion potentials can have a notable effect on the migration of the ions. The following cases are selected to illustrate some of the various possibilities which can arise.

(A) DIFFUSION POTENTIALS DUE TO CONCENTRATION GRADIENTS IN THE SOLUTION IN THE NEIGHBOURHOOD OF THE CRYSTAL.—It follows from the Nernst argument that any concentration gradient  $dc/dx$  in solution is accompanied by a potential gradient  $dE/dx$  in the solution if the ionic mobilities  $U^+$  and  $U^-$  differ. When a solution of a pure ionic crystal is considered with ions of equal valency  $n_e$ , the well-known expression is obtained for the potential gradient :

$$\frac{dE}{dx} = - \left( \frac{U^+ - U^-}{U^+ + U^-} \right) \frac{RT}{cF n_e} \cdot \frac{dc}{dx}.$$

Such a potential gradient in the body of the solution can be substantially modified by the presence of foreign ions whose valency and ionic mobility are very different from  $n_e$  and  $U^+$  or  $U^-$ . Three types of ionic impurity may be quoted which may be expected to have a substantial effect in modifying concentration gradient potentials in solution, and which may in consequence modify crystal growth.

(i) The  $H^+$  ion when the anion is common, and to a less degree the  $OH^-$  when the cation is common.<sup>1</sup>

(ii) Ions of high valency as an impurity in a solution of ions of low valency.

(iii) Colloids capable of acting as ions with very low mobility.

<sup>1</sup> cf. Abegg and Bose, *Z. physik. Chem.*, 1899, **30**, 545.

Before an assessment can be made of the role of such impurities in modifying crystal growth by modifying concentration gradient potentials, it would be of value to have an experimental technique which would reveal the equipotential surfaces around an ionic crystal growing in solution. Such a technique does not seem to be available at present. But studies of the effects of the deliberate addition of impurities such as (i), (ii) or (iii) may be useful in indicating the kind of distribution over a plane face, and around edges and corners of a growing crystal.

Alterations of the viscosity of the solution would also modify the concentration gradient potentials around a crystal, by affecting the values of  $U^+$  and  $U^-$ . Probably this effect is subsidiary in aqueous solutions until the change in ionic mobilities is substantial.

(B) THE POTENTIAL GRADIENT ACROSS THE INTERFACE BETWEEN THE LATTICE AND THE SOLUTION.—Even under conditions where growth rates are negligible, and concentration gradient potentials in the bulk of the solution can be neglected, a potential difference should normally persist across the interface between the crystal and the solution. For example, such potential differences should persist in saturated solution in equilibrium with a crystal surface.

Unequivocal theoretical calculations of the magnitude of this potential difference do not appear to be available.<sup>2</sup> The various physical factors contributing to this potential difference have not been fully elucidated, but its origin can be grasped by considering an ionic crystal in equilibrium with its very dilute vapour *in vacuo*. The steady state is somewhat more complicated for an ionic crystal than for a homopolar crystal in equilibrium with a monatomic vapour, owing to the fact that the work done against the crystal lattice forces on removing isolated positive ions is usually not quite the same as the work done in removing isolated negative ions, on account of differences in the polarizabilities and van der Waals' attractions. A potential difference between the interior of the solid and the vapour must be built up till the rate of vaporization of the two ions becomes equal. The way in which this potential difference is established need not be particularized here. One process would be a displacement of the ions near the surface of the crystal from their normal equilibrium positions.

A similar potential difference may normally be expected for crystals in contact with solution. Although the calculation of the magnitude of this potential difference in solution is not finally solved, it is important to consider how it would be modified at various crystal faces by the presence of foreign ions of the type considered under (A) above. Foreign ions especially of the types (ii) and (iii) are well known to influence streaming potentials and will have a corresponding effect on the surfaces of an ionic crystal in aqueous solution.

(C) CATAPHORESIS OF CRYSTAL NUCLEI IN SOLUTION.—Under conditions such that potentials described under (B) are sufficiently large, it should be possible to cause the crystal nuclei which are formed in solution to migrate to the electrodes by applying a potential gradient. It should be noted that such cataphoresis will be sensitive to factors which affect the surface potentials of the nuclei. Reference may be made to experiments to test this possibility.<sup>3</sup> In these experiments, by applying a potential difference across a pair of copper electrodes dipping in a supersaturated solution of copper sulphate, all the nuclei grow as crystals adhering firmly to the anode.

<sup>2</sup> cf. *Faraday Soc. Discussions*, 1947, 1, 3, 43.

<sup>3</sup> Ubbelohde, *Trans. Faraday Soc.*, 1940, 36, 863.

Owing to the incidence of the war, it has not yet proved possible to extend this experimental work. Some further discussion may, however, be given here in view of the statement<sup>4</sup> that such directed nucleation is *merely* due to the concentration differences at the two electrodes, arising from a passage of the current. In a typical experiment (Ubbelohde, loc. cit., p. 864) the initial concentration of copper sulphate corresponded with 66 g.  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  in 100 g. water at 20° C. The equilibrium concentration at the same temperature was approximately 32 g.  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  in 100 g. With electrodes of surface area 2.4 sq. cm., preferred nucleation on the anode was still definitely detectable after the passage of 0.2 mA flowing for 15 min., i.e., 0.18 coulomb.

This quantity of electricity would lead to an ultimate gain of  $\text{CuSO}_4$  in the anode region of

$$\frac{0.18}{96,500} \times 124.8 \times 0.625 = 1.45 \times 10^{-4} \text{ g.}$$

calculated as pentahydrate, using the transport number 0.625 for the anion.

Questions which arise are whether the gain of  $\text{CuSO}_4$  in the anode region can make a significant difference to the degree of supersaturation, and why all the crystals are found firmly adhering to the electrode, with evidence of preferred but not unique orientation.

The increase in concentration around the anode depends on the volume in which the gain of  $\text{CuSO}_4$  is contained. Considering this volume as a cylindrical sheath of thickness  $x$  around the anode, the increase in concentration is approximately  $6.0 \times 10^{-5}/x$  g./ml. For this increase to be an appreciable fraction of the supersaturation in the bulk of the solution (approx. 0.3 g./ml.)  $x$  must be of the order of  $10^{-4}$  cm. This thickness of anode layer is physically not unreasonable and the increased probability of nucleation in the anode region may well be attributed to the increased concentration resulting from the transport of electricity.

But mere increase in probability of nucleation does not explain why the crystals are found to adhere firmly to the anode, and with preferred but not unique orientation. This appears to be a significant observation for the mechanism of growth of ionic crystals. One explanation could be (Ubbelohde, loc. cit., p. 866) that the  $\text{SO}_4^{2-}$  ions discharged at the surface of the copper electrode can act as two-dimensional nuclei for the growth of  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ . The preferred but not unique orientation observed for the crystals would be analogous with oriented overgrowths in other cases. An alternative explanation is that the increased concentration due to the transport of electricity leads to a higher probability of nucleus formation in the anode region, and that these nuclei are swept to the anode by cataphoresis as soon as they are formed. Since the streaming potentials at different crystal faces are not the same, there would be a tendency to turn the nuclei in the current flow, which would explain preferred orientation.

Whatever the explanation, the phenomenon of the electrolytic growth of ionic crystals offers one of the problems of crystal growth which appear to be related with the potential distribution around ionic crystals.

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<sup>4</sup> Boerboom, *Nature*, 1947, **159**, 230.

## GENERAL DISCUSSION

**Dr. M. H. R. J. Plusjé** (*Geleen*) said : I should like to make a comment on the paper of Dr. Dunning concerning the kinetics of crystallization in solution. Dr. Dunning has grown crystals in a continuous manner under steady conditions. This is the way in which crystallization is carried out in practice on a large scale, and I am particularly interested in crystallization as a unit operation.

Dr. Dunning has found that the rate of linear crystal growth is a function of the supersaturation and Table I of his paper proves that small deviations in the supersaturation have a marked influence on the rate of growth.

In my opinion, however, the figure he uses for the supersaturation is not the actual supersaturation under which the crystals were growing, even after the correction made for the somewhat higher temperature of the solution caused by the heat of mixing. To find the supersaturation he has taken the difference between the actual concentration, determined by immediate filtration, and the concentration after a prolonged time of mixing, the last concentration after a correction for a small difference in temperature due to the heat of mixing. In determining the supersaturation he has supposed that the last-named concentration is the equilibrium concentration at the surface of the crystal.

My opinion is that this is not correct. The reason is that the temperature of a growing crystal is higher than the temperature of the solution in which it grows. This is caused by the heat of crystallization, which is released at the surface of the crystal. When crystallizing in a continuous manner under steady conditions the crystals reach a certain fixed temperature, which remains constant during the growing process. Therefore, there is a constant flow of heat (the heat of crystallization) from the crystals to the solution and from the solution through the wall of the container to the cooling medium (heat of crystallization plus heat of the solution).

We have tried to determine this difference between the temperature of growing crystals and the temperature of the solution. With an indirect method we found for  $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$  in an aqueous solution a difference of about  $1/10^\circ \text{C}$ : the exact value depending on the ratio between the total crystal surface and the surface of the cooled vessel.

The actual concentration of the solution at the surface of a growing crystal is therefore never the equilibrium concentration at the temperature of the solution, but somewhat higher. Because small differences in the supersaturation have such marked influences on the rate of growth, I think it absolutely necessary to take the "crystal temperature" and the heat flow into account in order to obtain a real picture of the kinetics of crystallization in solution.

**Dr. W. J. Dunning** (*Bristol*) (*communicated*) : Dr. Plusjé reminds us that the temperature of the growing crystal is higher than that of the solution. An estimate of this temperature difference is readily obtained if certain assumptions are made. As he says, under steady conditions of continuous crystallization the crystals reach a fixed temperature  $T'$  and if in addition the suspension is adequately stirred, the temperature  $T_0$  of the solution is also constant up to a small distance  $\delta$  from the surface. If the crystal is growing at a constant linear rate  $g$ , the rate of heat production at the surface can be derived. Part of this heat raises the temperature of the new growth to  $T'$  and the rest is conducted away down the temperature gradient  $(T' - T_0)/\delta$ . This model gives

$$T' - T_0 = \frac{g \Delta H}{M(cg - K/\delta d)},$$

where  $\Delta H$  is the heat of crystallization per mole,  $g$  the linear rate of growth,  $M$  the molecular weight,  $c$  the specific heat,  $K$  the thermal conductivity, and  $d$  the density. If we take the largest value of  $g$  in Table I ( $8.6 \mu/\text{min.}$ ), and assume  $K \sim 10^{-3}$  cal.  $\text{cm.}^2/\text{cm.}^\circ \text{C}$ , by using the Neumann-Kopp rule a value of  $c \sim 0.2$  can be estimated, we then find that with  $\delta = 10^{-4}$  cm.,  $T' - T_0$  is of the order  $10^{-4}^\circ \text{C}$ .

The figure of  $0.1^{\circ}\text{C}$  found by Dr. Plusjé can be explained if it is assumed that his stirring is less efficient than we have assumed to be the case in the discussion above. This would be consistent with his finding that the temperature difference depends upon the ratio of the areas of the crystal surface and the cooling surface.

In this connection we might mention again that particular attention was paid to stirring efficiency in our experiments. The efficiency used was such that on halving the speed of rotation of the stirrer no significant effect was noted.

Burton and Cabrera comment that it would be very interesting to develop experiments directed to avoid all possible foreign nuclei which could facilitate nucleation. Our continuous method does not lend itself to *exhaustive* elimination of growth nuclei, but the batch method does allow operation in a closed system by which nuclei can be progressively eliminated to an isolated part of the apparatus. Work is in hand on this latter technique but all the experimental problems have not yet been solved. In connection with our results for the continuous method, it is easy to show that since the reagents were drawn from the same bulks in all but the first three experiments of Table 1 and if it is assumed that the rate of introduction of foreign nuclei is proportional to the rate of introduction of the reagents (i.e., the number of foreign nuclei per unit volume of reagents is constant), then if the nucleation is not homogeneous but solely due to foreign nuclei, the product of  $n_0$  and the time of passage should be a constant. This is certainly not the case as inspection of Table 1 will show, and this lends support to the view that in these experiments the nucleation was homogeneous.

**Mr. W. K. Burton and Dr. N. Cabrera** (*Bristol*) (*communicated*): With reference to the papers by Dunning and others it seems to us worth while to point out again the essential quantitative disagreement between theory and experiment, in three-dimensional nucleation from solution. The theoretically expected critical supersaturations, for which nucleation should occur, are always of the order of a hundred times bigger than those experimentally observed. Of course, the theory requires the knowledge of the surface energy between nucleus and solution; the theoretical estimates are made taking some fraction of the heat of solution for this surface energy. The disagreement with experiment would disappear if the surface energy is assumed to be 20 or 30 times smaller than the estimate above.<sup>1</sup> We do not think that this estimate can be wrong by this factor.

This is essentially the same situation as that occurring in the surface nucleation. We tried several possible explanations (entropy factors, influence of the mobility of the adsorbed layer), but none changes the disagreement by an appreciable amount.<sup>2</sup> The difficulty has now been overcome by the dislocation mechanism proposed by Frank.

In nucleation from solution no explanation has been found. It would be very interesting to develop experiments directed to avoid all possible foreign nuclei, which could facilitate nucleation; it is possible that real three-dimensional nucleation has not yet been observed.

**Dr. R. F. Strickland-Constable** (*London*) said: Bransom, Dunning and Millard find that the rate of crystal growth appears to approximate to a linear function of the supersaturation. It is believed that this result can be rendered very probable on the basis of some rather general considerations which have no relation to any particular mechanism. For this purpose it is necessary to remember that crystal growth involves a balance between two other processes, namely, deposition and solution. Then the growth rate can be expressed as:

$$g = F(c) - K, \quad \dots \quad (1)$$

where  $g$  = net rate of growth;

$F(c)$  = gross rate of deposition of solute. It is expressed as an entirely unknown function of the total concentration (not as a function of the supersaturation);

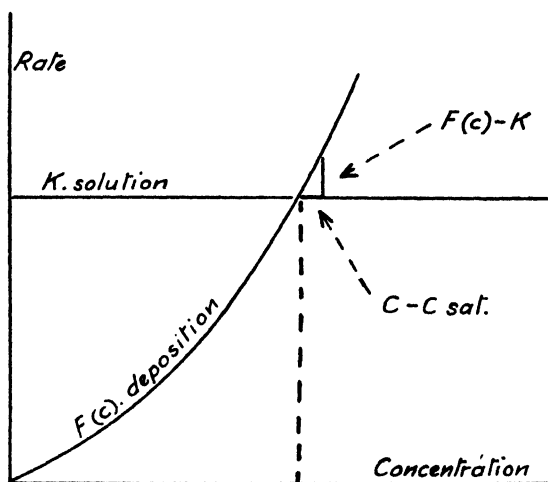
<sup>1</sup> Amsler, *Helv. phys. Acta*, 1942, **15**, 699; see also Dunning and others, *This Discussion*.

<sup>2</sup> Burton and Cabrera, *This Discussion*.

$c$  = concentration of the solution in contact with the crystal face ;  
 $K$  = gross rate of solution, which is a constant, independent of the concentration.\*

In the Figure the curve  $F(c)$  representing deposition rate and the straight line corresponding to the constant rate of solution  $K$  intersect at the point where  $c = c_{\text{sat.}}$ . The rate of crystal growth is shown as the difference in height of the two curves. It is clear from the small triangle that  $(F(c) - K)$  is directly proportional to  $(c - c_{\text{sat.}})$  to a close degree of approximation provided that :

- (1)  $F(c)$  is continuous in the neighbourhood of  $c = c_{\text{sat.}}$ ;
- (2)  $c - c_{\text{sat.}}$  is small compared to  $c_{\text{sat.}}$ .



The former condition is most probably fulfilled since there is no reason why the rate of deposition, which is here looked on as a property of the solution, should behave in a special manner at the point of crossing the solubility curve. The second condition is normally fulfilled for practical reasons. It is therefore possible to write :

$$g \approx k(c - c_{\text{sat.}}), \text{ true for } c - c_{\text{sat.}} \ll c_{\text{sat.}} \quad (2)$$

This principle has an important application in experiments such as those of Benvivoglio.\* This author found that in the case of a number of crystals the relative rates of growth of the different faces were constant, in spite of a variable degree of supersaturation.

The most general form of growth law which would express this result, considered by itself, is

$$g_A = k_A \{f(c) - f(c_{\text{sat.}})\}, \quad (3)$$

where  $g_A$  = net rate of growth of face A ;

$k_A$  = constant appropriate to face A ;

$f(c)$  = unknown function of the concentration of the solution in contact with the face. This function must be the same function for each face of the crystal,

with similar expressions for the other faces B, C . . . etc., whence  $\frac{g_A}{k_A} = \frac{g_B}{k_B} = \text{etc.}$

\* Benvivoglio, *Proc. Roy. Soc. A*, 1927, 115, 58.

\* Since the growth rate is known to be sensitive to small quantities of impurities the above equation must hold only for a given state of the solution containing a definite constant amount of such impurities.

In order that the condition,  $g = 0$  for  $c = c_{\text{sat.}}$ , may be fulfilled, where  $c_{\text{sat.}}$  is the concentration of the saturated solution,  $K$  must be equal to  $F(c_{\text{sat.}})$ , and eqn. (1) can therefore also be written as :  $g = F(c) - F(c_{\text{sat.}})$ .



Eqn. (2) is a special case of (3), in which the arbitrary function  $f(c)$  is placed equal to  $kc$ . If therefore the law expressed in (2) is a general law, the results of Bentivoglio are accounted for. At the same time the expression (3) has been written down in order to show that Bentivoglio's results are, considered by themselves, consistent with a more general law which allows the growth rate to depend on an arbitrary function of the concentration which, however, must be the same for each face. It seems more likely, however, that Bentivoglio's results are, in fact, due to the general validity of eqn. (2).

**Dr. W. J. Dunning** (*Bristol*) (*communicated*): Regarding some remarks of Prof. Juliard, I would make the following suggestion. Even in the case of homogeneous nucleation the crystals finally obtained are of approximately the same size, if as is usually the case the rate of nucleation depends on a higher power of the supersaturation than the rate of growth does. Then the nuclei born earliest when the supersaturation is greatest are not only the most frequent but become the largest crystals, hence a high proportion of the weight of the precipitate will be in this size group. Casual observation (as distinct from number distribution analysis) will give an impression of size homogeneity.

Again the sigmoid shape is not solely a characteristic of the presence of foreign nuclei. With homogeneous nucleation, the rate  $dS_\theta/d\theta = 0$  at the beginning and the end of the precipitation, but it is finite during crystallization, hence there must be an inflection point.

If growth occurs only on foreign nuclei and homogeneous nucleation does not occur, the eqn. (7a) in Bransom and Dunning's paper takes a simple form, from which the relation

$$\frac{d(S_\theta - S_0)^{1/3}}{d\theta} = \frac{\omega d}{M} \cdot n_0 f(S_\theta)$$

can be obtained, where  $n_0$  is the number of foreign nuclei per unit volume. In a series of experiments of different initial  $S_0$  and  $n_0$ , the left-hand side can be obtained for each and plotted against  $S_\theta$ . Then the ratios of the ordinates for all  $S_\theta$  values ought to be constant and equal to the ratios of the  $n_0$ 's. From these plots the functional dependence of  $f(S_\theta)$  can be obtained apart from a constant factor.

**Dr. S. Fordham** (*Stevenston, Ayrshire*) said: The results in my paper showed that there was a strong probability that strained crystals of ammonium nitrate had an initially increased rate of growth. More recently the surfaces of typical crystals have been examined by a replica technique with the electron microscope. Fig. 1 shows the surface of an unstrained crystal characterized by what appear to be cracks parallel to the (001) plane of the crystal. Fig. 2 shows that straining of the crystal causes small areas to be raised above the general level, thus producing irregularities of finite size. The two photographs were taken by Mr. J. Ames.

**Dr. F. C. Frank** (*Bristol*) (*partly communicated*): With reference to Dr. Fordham's observations, for reasons given, I should not expect the change in dislocation content produced by straining the crystals to cause a significant change in the rate of growth (though it could affect the critical supersaturation for growth, if one were found). In this case, I think the transient extra growth is to be explained more on Prof. Stranski's lines, by completion of layers from the steps produced by slip. This remains true although the slip steps are shown by electron-micrography to be of a rather complex character. Fordham refers to them as "dislocations." The word "dislocation" has acquired a very definite meaning in the theory of the solid state, and ought not to be applied freely to any sort of derangement of a crystal: though various sorts of derangement can be analyzed into systems of dislocations.

Prof. Stranski has mentioned the high rate of growth of twin crystals having faces which meet in the composition plane so as to make a re-entrant angle with each other. A notable mineralogical example of this is fluorite. The great majority of large fluorite crystals exceeding, say, 1 cm. in size, especially those of Weardale which make up the principal exhibits of fluorite in British museums, are interpenetrating twins, with a twin emergent on every face. Exceptions to

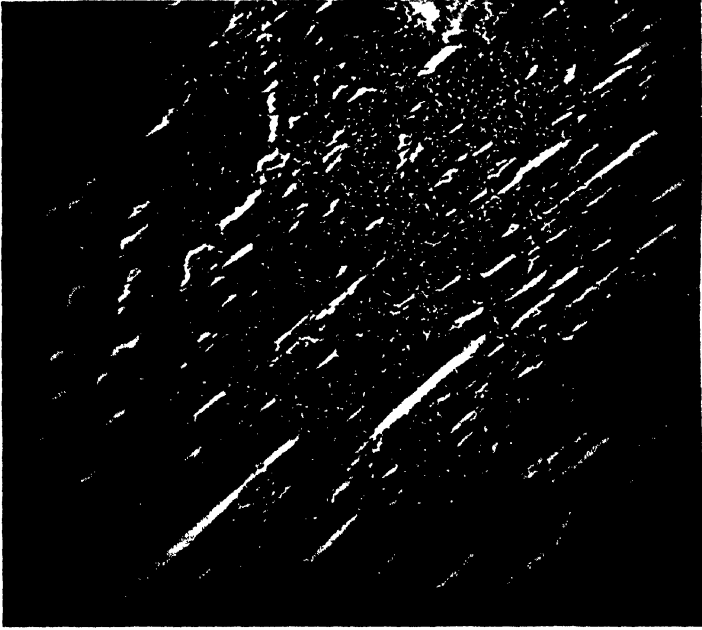


FIG. 2.



this rule always have\* visible disoriented blocks. Sometimes the corner of the twin is just on the point of being submerged—evidently growth then ceases. Each face of these twin crystals shows a pronounced growth pyramid of unusually steep vicinal faces centred on the common line of the twin faces which meet on the composition plane. In a typical example (of which a lantern slide was shown) the inclination of these faces to the (100) face is  $3.0^\circ$ , in contrast with  $20'$ , which is the most which is usually observed for growth pyramids centred on some ordinary point in the face of a crystal, as observed on alums, for example, by Sir Henry Miers.<sup>4</sup> This unusually steep growth-pyramid signifies an unusually high rate of emission of growth fronts from the initiating centre (in this case the junction with the twin) compared with their rate of travel over the surface—of the order 10 times as great as when the initiating centre is a simple dislocation or group of dislocations.

Another admirably simple example to be seen in collections of minerals is provided by calcite; the "heart-shaped" or "butterfly-shaped" twins of calcite on (110) are characteristically 10 times as large, linearly, as the accompanying population of single crystals.

With regard to the visible growth steps or layers to which Dr. Bunn draws attention, I certainly think that at least in some cases they arise in a manner which Prof. Becker aptly likens to the formation of shock-waves; i.e., that for some reason molecular steps bunch together. We have then to find the influence which leads to this bunching. This must be a "second-order" effect: for if deposition occurs at equal rates on every molecular step-line, the surface profile is conserved, travelling with the steps; while if there is competition between the steps so that their rate of accretion is proportional to the distance between them the profile is conserved in the mean, while the molecular steps travel through. Neither of these extreme conditions, nor any simple linear combination of them, gives rise to an accentuation of irregularities in the profile; this requires that the leading members of a group should travel slower than the rest. This will tend to occur in a stirred or convecting medium once the height of the multiple step becomes an appreciable fraction of thickness of the laminar diffusion layer at the surface of the crystal; but this effect will be very slight until a substantial bunching has occurred, and I would attribute the initial bunching to fluctuations in concentration at the initiating centre.

The most important point to notice, however, is that with this sort of interpretation there are no layers, only a stepped profile. I suppose that there are also other types of crystal growth with a genuine lamination; but one must avoid confusing the two, taking the visible steps as certain evidence of real layers. In particular, steps which increase in height as they spread outwards cannot correspond to layers.

**Dr. S. Fordham** (*Stevenston, Ayrshire*) said: Dr. Frank has suggested that my results were due to crystallization on slip planes in the strained ammonium nitrate crystals. Neither optical nor electron microscopic examination indicated the presence of regular slip along glide planes. On the contrary, crystals had irregularities in their surface which were of finite size and in appearance seemed to be very similar to the dislocations discussed by Dr. Frank although they were, of course, on a larger scale. While willing to avoid the use of the term dislocation in describing these irregularities, I think this is a matter of nomenclature which does not affect the interpretation of the results.

**Prof. W. E. Garner** (*Bristol*) said: The films of Dr. Bunn and Emmett showed the spread of crystallization as a series of waves starting at some point near the centre of the crystal surface. Crystal growth therefore appears to be a periodic phenomenon. In these experiments the supersaturation was probably high, and at high supersaturations the formation of a two-dimensional nucleus at the centre of a crystal face will have a high probability, even if no dislocations be present. The supersaturation at the centre of a face will decrease when a nucleus is formed, and increase again as the nucleus grows away from its point of origin. Therefore a periodic formation of nuclei implies a periodic change in supersaturation. Likewise the probability of nuclei formation will vary periodi-

<sup>4</sup> *Phil. Trans. A*, 1904, **202**, 459.

cally as the supersaturation fluctuates. It is possible, therefore, that the phenomena observed are due to the interrelationship between the probability of nuclei formation and the supersaturation. It appears to be important to work out the dynamics of such processes. In crystallization from melts, a periodic fluctuation in the temperature of the melt in the neighbourhood of the crystal surface is probably the effective agent in creating the wave motion.

**Dr. K. G. Denbigh** (*Cambridge*) said: I would draw attention to the fact that crystals are occasionally found in which there are a number of liquor inclusions situated symmetrically with respect to the centre of the crystal. During the war this had been observed both in R.D.X. and in hexamine. It seems that the mechanism depends on the formation of a symmetrical dendrite at an early stage of growth. The process by which R.D.X. crystals are formed had been carried out under the microscope and it was seen, a few seconds after initiation, that minute cross-shaped dendrites were formed. At a later stage of growth these developed into crystals of a more regular shape and the symmetrical inclusions were due to the trapping of mother liquor at the angles of the cross. In a particular case there were twelve liquor inclusions in a hexamine crystal situated with almost perfect symmetry about its centre. It was of interest that these inclusions were almost spherical and did not show the plane faces of the crystal.

Why is the structure of snowflakes so remarkably symmetrical? During a hard winter I have observed an ice crystal growing on the surface of still water in a bath. Over a period of a few days the dendritic crystal grew to the size of a plate, and its intricate pattern was perfectly symmetrical about the centre, like a greatly magnified snowflake. It had the usual hexagonal form and the question arose how it came about that *each of the six spikes* of the structure had exactly the same fernlike pattern. It was known that between one snowflake and another there were a great variety of patterns and it was therefore surprising that each of the spikes, in any one crystal, should develop in the same way. It seemed as if the pattern was controlled from the centre, as a chromosome controls the structure of a cell. It was perhaps related to Prof. Garner's point concerning periodic waves of crystallization radiating from a central point.

**Dr. W. A. Wooster** (*Cambridge*) said: Dr. Bunn has pointed out that some faces of a given crystal grow quickly while others grow more slowly or not at all. The concentration is greater near the non-growing surface than it is near the rapidly growing one. It may, therefore, be necessary to look for a cause which arises within the crystal rather than in the solution. I wish to put forward tentatively a suggestion based on the thermal motion of the atoms.

For an ionic crystal such as NaCl the amplitude of vibration of the ionic centres at room temperature is of the order of  $1/10$  Å, i.e., a small, but not negligible, fraction of the distance apart of neighbouring atoms. At a growing surface this amplitude of vibration may be greater owing to the unsymmetrical nature of the environment—solvent molecules on one side and regularly arranged atoms on the other. This atomic movement may determine the ease with which atoms can be attached to the surface.

The study of diffuse thermally scattered X-rays has shown that atomic movements, though random so far as any one atom is concerned, may be resolved into a series of waves of different frequencies travelling with the speed of sound. These waves constantly passing to and fro in the crystal will be reflected from the boundaries, and the amplitude of vibration at any corner, step or other discontinuity will be greater than on a corresponding flat surface. Thus if a crystal face has grown perfectly flat, and has no growing centres or steps, the elastic waves will be reflected but not scattered and the vibration of an individual atom in the surface may therefore be a minimum. On the other hand, if a crystal face has a step there may be a concentration of elastic vibrational energy just within the step which may keep the amplitude of atomic vibration greater than normal. This condition may favour further deposition and keep the step advancing.

A feature of growth, which, though not fully established for ionic crystals growing in solution, is certainly established for growth from the vapour, is the migration of atoms from the centre of a face to the growing edge. May it be

that this feature is also explained by the thermally generated elastic waves? If a step occurs on a surface there is a possibility that if vibrational energy is concentrated within the step it may act like a pulsating membrane and pump the liquid along the surface from the centre of the face towards the edge.

The question was also raised as to what mechanism could determine the symmetrical nature of the pattern of an ice crystal growing in still water, i.e., why all the branches arise on the opposite sides of a given stem at just the same distance from the centre. In a stem growing out in opposite directions from a centre, there will be elastic waves which will have the same vibrational pattern at the same distance from the centre on either side. If the nodes and antinodes of the elastic vibration pattern determine the generation of the branches, then the branches would occur at the same distance from the centre.

**Dr. D. R. Hale** (*Cleveland, Ohio*) (*communicated*): Bunn and Emmett call attention to the rounded surfaces of growth on high-index faces. The (001) face or basal plane in quartz is not a natural face and would thus be the equivalent, so far as growth is concerned, of a high-index face on, e.g., sodium chloride. In the work at The Brush Development Co., Cleveland, Ohio, on quartz crystal growing we have noted a high rate of growth, yielding rounded surfaces, on the artificial (001) face obtained by sawing the crystal.<sup>5</sup> The rate of growth is about an order of magnitude faster than that on a rhombohedral face. These observations may be added to those mentioned in the paper as evidence of the indiscriminate, high-rate deposition on high-index surfaces. A further common type of deposition observed on the artificial (001) face of quartz is an assemblage of minute, oriented trigonal crystals growing in the *c*-direction and fused with their neighbours at sufficient edges so that a porous structure results. The separate crystals in this growth generally terminate in trigonal caps, and no rounded points or areas are produced.

Thirty synthetic quartz crystals have been examined for evidence of layer formation. About a third of them do not have sufficiently plane faces to show unmistakable evidence of layer growth. On a few of the reasonably flat surfaces a regular pattern of fine and closely spaced concentric lines is evidence of the growth mechanism described by Bunn and Emmett. Many of the well-developed crystalline faces, particularly the rhombohedral faces, exhibit low rounded domes frequently outlined in a number of what seem to be contour lines, so that the appearance from above is that of looking at a map and seeing a hill marked on by lines of constant level. These lines are assumed to be the steps from one growing level to another, but in these quartz crystals the edge seems always to fall away in a sharp concave surface which hardly levels out before the next contour line is reached. This appearance seems to indicate that growth is taking place on faces of high index.

**Dr. F. C. Frank** (*Bristol*) (*partly communicated*): Bunn and Humphreys-Owen have produced some delightful experiments demonstrating that crystal growth is a structure-sensitive process. I am disappointed that they should finish their accounts of these phenomena by saying they are puzzling. Such things as a sudden change in growth rate are to be expected. They could arise from a sudden rearrangement of dislocations (since the ability of dislocations to move under small stresses is one of their fundamental properties). They can also be produced by adsorption of a very small amount of impurity on the step-line connecting a dominant pair of dislocations. These observations do seem to suggest, however, that in these particular experiments the number of dislocations influencing growth may be quite small.

Let me now deal with the "Berg effect," firstly pointing out what a very odd effect it is. It is not at all similar to what was observed by Volmer and Estermann. In their experiment a crystal of mercury grows in the form of a very thin plate from the vapour at low temperature in a vacuum. The mean free path is about 10,000 times the size of the crystal. It is found that every molecule which strikes the crystal anywhere on its surface sticks, but migrates and is built into the crystal only at the edge of the plate. This surface migration is entirely understandable and just what we ought to expect.

<sup>5</sup> Hale, *Science*, 1948, 107, 393.

In the experiment of Berg, Bunn and Humphreys-Owen, on the other hand, we have a crystal growing from solution and its size is about 10,000 times the mean free path in the surrounding medium. Ions migrating over the surface of the crystal will suffer jostling from the molecules of the surrounding medium. It appears quite possible, in the circumstances, that migration over the surface will not be observable in comparison with diffusion in the solution. But suppose it were: if there were a very mobile layer at the crystal surface, the boundary condition at that surface would be an "equipotential" one, and, like electrostatic lines of force, the lines of diffusion flow would be most concentrated at the crystal corners. If there is no special surface migration, a crystal preserving its form must have uniform deposition over the surface. But what is supposed to be observed by Berg and Humphreys-Owen is an excess of flux in the middle of each face. This is (as Berg knew) no ordinary layer of high mobility. It has a negative resistance. Diffusing matter goes out of its way, through a longer path in the ordinary medium, so as to reach the corners roundabout through this surface layer. This is not impossible, but is sufficiently odd to demand very good evidence before it is accepted.

Berg's evidence was two fold. A test which he only used qualitatively, in which he used the angles at which optical fringes met the crystal boundary to show that the normal gradient of concentration of solute ( $\partial c_a / \partial n$ ) was not constant (as he supposed it should be in uniform deposition without surface migration); and the more elaborate method of calculating concentration at many points in the medium, constructing a concentration map, and deriving ( $\partial c_a / \partial n$ ) at the boundary from this. He was wrong to suppose ( $\partial c_a / \partial n$ ) would be constant in the simple case. This would be true in dilute solution, but in more concentrated solution it is obviously necessary to allow for the fact that a part of the material required to build the crystal is there already, and a larger flux is needed where the solution is weakest. One may alternatively think of the necessary diffusion of solvent *away* from the crystal, which must also be greatest where the solution is weak. Then the boundary condition is

$$(\partial c_a / \partial n) = (w/D) (\rho_A / \rho_a) (\rho_a - c_a)$$

where  $w$  is the rate of advance of a crystal face (cm./sec.),  $D$  the diffusion coefficient (cm.<sup>2</sup>/sec.),  $\rho_A$  the crystal density (g./cm.<sup>3</sup>),  $\rho_a$  the effective density of the solute in solution (=  $\rho_A$  if there is no change of volume on solution) and  $c_a$  is the concentration of solute in the solution in contact with the crystal surface. Since the latter is about half the crystal density, and varies by 5 % or so over the crystal face, the correction is not negligible. However, according to data with which Dr. Humphreys-Owen provided me, it only accounts for about 20 % of the observed variation of ( $\partial c_a / \partial n$ ) across a crystal face.

Some uncertainty arises from the possibly illusory position of the crystal boundary, since lateral resolution in the microscope is necessarily sacrificed in compromising with the "parallel light" requirement for multiple-beam interference fringes. But the chief source of error is probably convection, the presence of which will invalidate the assumption that the flux of solute is simply proportional to the concentration gradient. Convection must occur because of the large gradient of density associated with the concentration gradient near the growing crystal. One may readily show that the ratio of the resulting convective transport to the diffusive transport is proportional to

$$gh^4 |\text{grad } \rho| / \eta D$$

where  $g$  is the acceleration due to gravity,  $h$  is the thickness of the cell in which the observations are made,  $\rho$  is the density and  $\eta$  the viscosity of the solution and  $D$  the diffusion coefficient of solute. By a rather crude estimation of the numerical factors involved it is found that the convective transport and diffusive transports are of similar order of magnitude when this dimensionless quantity is about 2000. In the experiments of Berg, Bunn and Humphreys-Owen we have typically:  $\text{grad } \rho = 5 \text{ g./cm.}^3 \text{ per cm.}$ ,  $\eta = 10^{-2}$  and  $D = 10^{-5} \text{ c.g.s. unit}$ , while  $h = 1 \text{ to } 2 \times 10^{-2} \text{ cm.}$ ; so that the above number varies from 500 to 8000. The effect of convection is thus never negligible in the experiments as conducted up to now, but since the fourth power of the cell thickness appears in the criterion, it is relatively easy to make the convection negligible by using

a thinner cell : let us say, 10 times thinner. Dr. Humphreys-Owen tells me it is practicable to use a supersaturation ten times as large as in most of his experiments so that the optical sensitivity remains the same. We shall then be able to see whether the Berg experiment really does exist.

It is perhaps worth making a few more comments on the design of the experiment. Time and expense appear to be the only considerations really favouring a micro-experiment. Removal of heat and suppression of convection are both achieved by using a thin cell, which is also advantageous for optical resolution. An increase of the lateral dimensions of the crystal and field of view is purely advantageous so far as these considerations are concerned, provided that half-silvered optical flats of sufficient area are available, but can only be achieved by growing the crystal *in situ*, which takes a time proportional to the area, or longer.

**Prof. A. Julliard** (*Brussels*) said : May I suggest that if there were any impurities in either solution or in the gas phase from which a crystal is formed this crystal may not grow at all ?

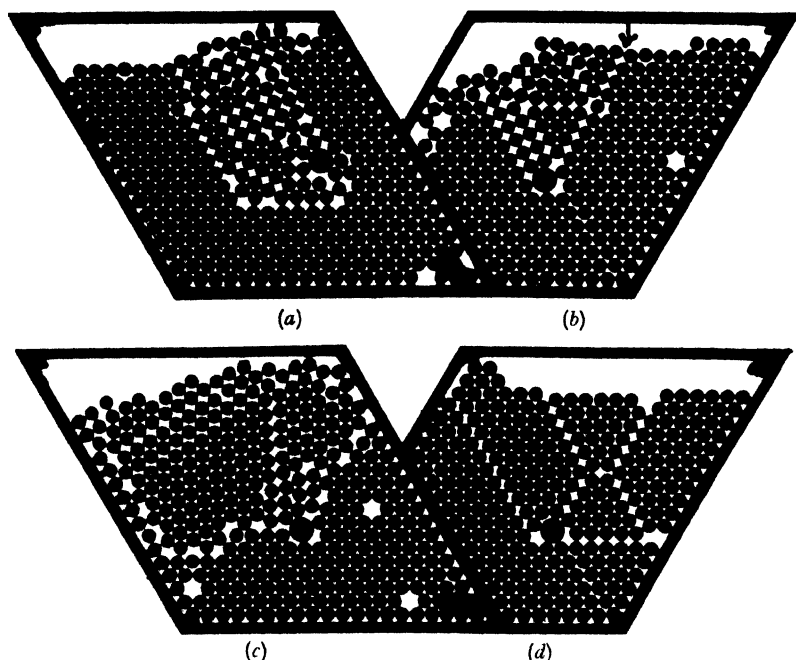


FIG. 1.—Perturbation of the crystal structure due to the imbedding of a single foreign particle : (a) local dislocation ; (b) centre of a helix molecular terrace formation ( $\downarrow$ ) ; (c) twinning formation ; (d) mosaic structure formation.

Foreign particles adsorbed on the surface of a crystal may exercise different effects.

(i) If these particles are strongly adsorbed and are present at a relatively high surface concentration they may prevent a further aggregation of the constituents of the crystal and so stop the growth of this surface. When this action is exercised on each surface of the crystal, the presence of this impurity can completely inhibit the precipitation. When this inhibiting action is only effective on certain surfaces, the impurity may simply modify the habit of the crystal.

(ii) If the particles are strongly adsorbed but present at a relatively low surface concentration they may be embedded in the crystal by its later growth. The presence of these foreign particles in the lattice may distort the lattice or introduce dislocations on a molecular scale which may be the origin of helix molecular terraces, of the twinning habit, or of the mosaic structure of certain crystals (Fig. 1).



(iii) If the particles are weakly adsorbed they may act as mineralizers in the broad sense of the word. With convenient geometrical and chemical conditions these particles can initiate on a crystal surface an active spot which acts as a step from which a new molecular layer of the crystal may grow. When, in addition, these particles are easily expelled from the newly formed layer, one can imagine that these growing particles may be pushed ahead on a step-shape protuberance on the surface of the crystal. Such protuberances could be the origin of those "multi-growth" layers whose existence was evidenced by the remarkable film on crystal growth shown by Dr. Bunn and Emmett.

**Mr. A. E. Robinson** (*Holton Heath, Poole*) said: The crystal habit of  $\text{Li}_2\text{SO}_4$  has been found to be considerably influenced by pH of the growing solution: at pH above 7 growth along  $x$  and  $y$  axes is encouraged: at pH below 5 growth along the  $z$  axis is encouraged. The growth appears to be somewhat slower at low pH. There is another effect of pH which may throw some light on the anomalies of the adsorption effects discussed in the previous section. Small amounts of phosphate (5 parts per million) have been found to inhibit growth at one polar end of the crystal. At the higher pH this ion is deposited on the crystal; at the lower pH it remains in the solution.

The addition of a surface-active agent to this solution is an attractive idea as one of the problems is the adherence of air bubbles to the growing crystal. These may persist throughout growth and result in a hole through the crystal. One wetting agent used prevented this effect, but crystal growth was rather slower and two extra faces parallel to the  $y$  axis were developed.

**Mr. L. J. Griffin** (*Egham*) said: It may be relevant to mention the part which can be played by the study of the surface topography of crystals using multiple-beam interferometric techniques as developed by Tolansky. By this means one can study natural and cleavage faces, and also synthetic growths on either of these types of faces, with a "resolution" in depth approaching molecular dimensions. One is thus enabled to arrive at a picture of the mechanism of growth of many crystals, and in particular many minerals, which are not otherwise amenable to study.

In order to illustrate the possibilities of the technique I should like to mention some work I have done on beryl with particular relation to Bunn's results given earlier in the Discussion. Such naturally occurring crystals have grown under unknown conditions, possibly with several complicating factors influencing their growth. Therefore in all work of this nature a guiding principle has been that several, and preferably many, crystals should show the same type of behaviour before any general type of behaviour is claimed. Several specimens have been found to show an extensive layer structure, the thickness of the layers varying between some hundreds down to three or four unit cells. These layers tend toward perfect conformity with the symmetry of the face, the conformity in general becoming more rigorous as the layers become very thin. It may be mentioned that the outline of these very thin layers shows no trace of the presence of dislocations of the type proposed by Frank. The importance of the nature of the layer edges has already been stressed by Bunn and it is worthy of note that multiple-beam interferometry provides a means, with beryl, of indexing the edges of the thickest layers. Some data have already been obtained but have not yet been numerically evaluated. Bunn's thesis of high index edges would, however, seem to be borne out. The nature of the layer edges on beryl is actually such as to produce a diffraction effect rendering them visible, under the microscope, even when only some four or five unit cells high. The limit of sensitivity of this surprising effect has not yet been capable of determination although evidence has been obtained for the observation of layers three unit cells thick. By utilizing this diffraction effect and interferometric methods, direct experimental proof has been obtained that the vicinal faces of beryl consist of extensive series of stepped layers. The growing points are sited, in general, towards the centre of the face and away from edges or corners. The observations on this point are not yet sufficiently extensive as compared with Bunn's to enable one to claim a general behaviour. In conclusion, it may be mentioned that the existence of layers has been observed on a number of other crystals, and in fact there seems little doubt that many crystals do grow by layer deposition.

**Prof. I. N. Stranski** (*Berlin*) said: The problem of the occurrence of visibly thick layers on growing crystals has two aspects. It is not sufficient to show that the thicker layers (or multimolecular lattice planes) extend more slowly than the elementary lattice faces (which are afterwards caught up by the lattice faces which begin later). It must also be explained why the thicker layers may not become thinner by escape of individual lattice planes from the base. A special mechanism of coarsening which sometimes occurs on single faces of metal crystals and which is obviously connected with the edges of the faces<sup>a</sup> may be mentioned in this connection.

I should also like to point out the fundamental difference between the growth and reduction of crystals of urotropine at low and high temperatures and also the remarkable variation shown by layer growth on faces of Cd or Zn crystals according to whether they are surrounded by the fused liquid or the gas phase (*Eisenloeffel*).

**Dr. C. W. Bunn** (*I.C.I., Plastics*) said: In answer to Dr. U. R. Evans' remarks on dendrite formation, I would like to give some additional details of the calculations of the diffusion process round a square crystal plate which are mentioned in my second paper. Only the results of long-continued diffusion (i.e., arrival of excess solute at face centres) are mentioned in the paper, because these appear relevant to the phenomenon of layer formation at face centres. But at the beginning of the process, corresponding to the early growth of a crystal nucleus, the reverse result is obtained—more solute arrives at corners than at face centres. This is the expected result, and may be regarded as due to the convergent diffusion flow to the corners, when diffusion has just started and the diffusion field does not extend far from the crystal. It is natural to suppose that dendrite formation may be due to this excess arrival of material at the corners of a polyhedral crystal nucleus—excessive deposition takes place on the corners, which begin to shoot outwards. But I should like to point out that the effect might be due either to this convergent diffusion effect, or to the fact that the supersaturation is higher at the corners than elsewhere; we ought to distinguish carefully between these two possible causes. I do not know of any evidence on the question which is the dominant effect; but since both work out in the same direction, we do not lack an explanation of dendrite formation! Moreover, once dendritic growth has started, not only does the growing tip retain the advantage of being in contact with the most highly supersaturated solution, but also the diffusion field will become organized to supply solute so as to continue the process by very convergent diffusion flow to the growing point. In fact, the difficulty is to explain why any crystals ever avoid dendrite formation and grow as polyhedra. That many of them do is presumably due, as Dr. Evans says, to the tendency towards the setting up of surfaces of lower surface energy—"healing," as I have called it. The results in my paper are those which follow if it is assumed that the crystal does avoid dendritic growth and that the diffusion field extends far out into the solution.

Dr. Wooster's suggestions on the possible influence of the thermal wave-pattern in the crystal on the growth of layers are very interesting and worth bearing in mind; but I do not agree that to explain the remarkable variations in growth rate of NaClO<sub>3</sub> crystal faces it is *necessary* to look for a cause which arises in the crystal rather than in the solution, the cause *may* be either in the crystal or the solution, and in my paper I have suggested particular solution conditions—i.e., variations of concentration gradient (not concentration itself) brought about by convection currents or other effects. Dr. Wooster states that "the gradient of concentration is greater near to the non-growing surface than it is to the rapidly growing one"; but, as far as our measurements go, this is not so—the actual concentration is greater at the non-growing surface, but the gradient normal to the face is less steep than at the rapidly growing surface, as one would expect; the normal gradients are roughly proportional to the rates of growth. It is true that we can only measure concentrations up to within, say, 10<sup>-3</sup> cm. of the face, and there might be sudden changes at very small distances from the face; but any sharp bend in the concentration-distance

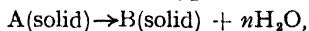
<sup>a</sup> *Optik*, 1948, 3, 17.

curve would only be persistent if there is a big change of diffusion constant near the face; this would mean a change of structure of the solution near the face. The ordered structure of the crystal might well affect the structure of the solution and its diffusion constant at very short distances (say, 10 Å); but it does not seem possible to measure concentrations at such small distances.

For the present all we can do is to measure concentrations as close to the face as we can get, and draw what conclusions we can on the provisional assumption that these measurements represent concentrations "at the face."

**Prof. W. E. Garner** (*Bristol*) said: With reference to Hartshorne's paper, and particularly to the high-temperature independent factor required (eqn. (10)) to account for this factor, the author, adopting a theory of Mott's, suggests that approximately  $10^7$  molecules of monoclinic sulphur, forming a mosaic block, are converted to rhombic sulphur by a trigger action.

Discrepancies in the temperature independent factor of a similar character are found in certain solid reactions of the type



where nuclei of B are formed in the surface of crystals of A simultaneously with the liberation of water. Where the nuclei of B have pseudo-crystalline shapes and presumably grow layer by layer, as in crystallization from the melt or from solution, the Polanyi-Wigner equation,

$$\text{rate} = \nu N e^{-E/RT},$$

gives the normal value for  $\nu$  of  $10^{13}$  and  $E$  is the same as  $Q$ , the heat of dissociation of the solid.  $N$  is the number of water molecules per sq. cm. of interface. In these cases there is very close coupling between the formation of the new and the destruction of the old phase, no activation energy being needed in excess of the heat of dissociation. On the other hand, chrome alum gives spherical nuclei and layer growth obviously does not occur. In this case the rate of growth of nuclei of solid B is given by

$$\nu = 10^{25} N e^{-31,000/RT}.$$

There is thus a discrepancy of  $10^{12}$  in the frequency factor and a further anomaly is found, namely, that  $E$  is no longer the same as  $Q$ , which is 16 kcal. These abnormalities can be accounted for if chrome alum possesses a mosaic structure and if the reaction spreads at the normal rate within a mosaic block, but needs a high activation energy to penetrate adjacent blocks.

In parallel with the case of chrome alum, it is suggested that for rhombic sulphur the rate of conversion within a mosaic block might be given by  $\nu N e^{-q/RT}$ , where  $q$  is the free energy of transition from monoclinic to rhombic sulphur. Since  $q$  is small, transition within a mosaic block will be very rapid. The  $E$  that is measured would then be the activation energy required to form nuclei of rhombic sulphur between adjacent mosaic blocks.

**Dr. W. J. Dunning** (*Bristol*) (*communicated*): Dr. Hartshorne has given an interesting interpretation of his experimental results, but there is another point of view worth considering. Accepting his model that there is a thin layer one molecule thick which has properties similar to a gas, and which is situated between the two crystalline forms, the rate of growth of the lower temperature form should be given by Volmer's equation. The process would then be very similar to the growth of a crystal from a supersaturated vapour of pressure  $p_1$ , this vapour pressure would be given by

$$p_1 = C e^{-E_v/kT},$$

where  $C$  is a constant and  $E_v$  the latent heat of evaporation of the high temperature form. Crystal growth from the vapour requires two-dimensional nucleation which contributes another term to the activation energy.

With

$$g = w_1 F \chi \delta \frac{\mu_1 - \mu_{1\infty}}{kT} \cdot e^{-A''/kT} \cdot e^{-A'/kT},$$

where  $g$  is the rate of growth; putting  $\chi = \frac{\rho \delta}{\mu_1 - \mu_{1\infty}}$ , and neglecting the term in  $A'$ , we have

$$g = \text{const.} \cdot e^{-E_v/kT} \cdot e^{-A''/kT}.$$

but 
$$\frac{A''}{kT} = \frac{\omega M \rho^2 N}{2d\delta R^2 I^2 (\mu_I - \mu_{I\infty})},$$

and 
$$\mu_I - \mu_{I\infty} = \frac{q}{I} \cdot (T_0 - T),$$

we obtain 
$$\log g = \text{const.} - E_0/kT - \frac{\text{const.}}{I(T_0 - T)}.$$

Applying this equation to Dr. Hartshorne's results, it is found that

$$\log V = 38.77 - \frac{20,200}{RT} - \frac{35,000}{I(T_0 - T)}$$

fits them quite well.

**Mr. Y. Haven** (*Eindhoven*) said: I would propose an alternative mechanism for polymorphic transformations and recrystallization processes which avoids the conception that one atom catalyzes, e.g.,  $10^7$  other atoms, as has been proposed to account for the large pre-exponential factor. Between the two phases a boundary region showing a certain amount of disorder is assumed. At a given moment only some of the atoms in this region are in a position to move; the equilibrium concentration is given by  $A' e^{-E'/kT}$  ( $E'$  = energy of disorder) and the mobility by  $A'' e^{-U/kT}$  ( $U$  = activation energy for transition).

So the transformation velocity may be written as (apart from other factors)

$$v \propto A' e^{-E'/kT} \cdot A'' e^{-U/kT},$$

where  $E = E' + U$ .

Both  $A'$  and  $A''$  may contain large entropy factors  $e^{+S/K}$ . An entropy factor of  $10^2$  in  $A''$  may be a reasonable one, so if the pre-exponential factor is  $10^7$  times greater than has been expected  $A'$  should contain an entropy factor of the order of magnitude  $10^6$ . This may be compared with ionic crystals where an entropy factor of  $10^4$  in the expression for the degree of disorder is very common, e.g., the number of vacant lattice sites in LiF is given by

$$n/N = 10^4 \cdot e^{-16,000/T},$$

where  $N$  and  $n$  = number of lattice sites and vacancies per  $\text{cm.}^3$  respectively.

**Dr. W. J. Dunning** (*Bristol*) (*communicated*): Prof. Davies and Mr. Jones interpret the turning points on the curves in their Fig. 3 as giving the concentration of the metastable limit. That this is correct can be seen from the following argument. The equation

$$S_\theta = S_0 - \frac{\omega d}{M} \int_0^\theta F(S_t) \cdot \left\{ \int_t^\theta f(S_\tau) \cdot d\tau \right\}^3 dt$$

gives 
$$\frac{dS_\theta}{d\theta} = 3f(S_\theta) \cdot \int_0^\theta F(S_t) \left\{ \int_t^\theta f(S_\tau) d\tau \right\}^2 dt,$$

$dS_\theta/d\theta$  is their ordinate ( $y$ ) in Fig. 3 and  $-S_\theta$  is their abscissæ ( $x$ ). Hence

$$\frac{dy}{dx} = -3 \frac{df(S_\theta)}{dS_\theta} \cdot \int_0^\theta F(S_t) \left\{ \int_t^\theta f(S_\tau) d\tau \right\}^2 dt - 6 \left\{ f(S_\theta) \right\}^2 \cdot \int_0^\theta F(S_t) \int_t^\theta f(S_\tau) d\tau dt.$$

Their turning point is presumably where  $dx/dy$  is changing very rapidly. The only quantity on the right-hand side which is changing rapidly is  $F(S_t)$  and the point where it is changing is the metastable limit.

**Mr. E. O. Hall** (*Cambridge*) said: I should like to draw Dr. McCrone's attention to similar results in two papers by Boas and Honeycombe<sup>7</sup> where similar grain boundary migration problems are studied in non-cubic metals, and the cause is traced to the strain set up in the matrix by anisotropic expansion during the thermal cycles.

<sup>7</sup> Boas and Honeycombe, *Proc. Roy. Soc. A*, 1946, **186**, 57; 1948, **188**, 427.

**Dr. H. K. Hardy** (*Stoke Poges*) said : Reference has been made to some experiments on crystal growth as influenced by an electric potential. The same principle has been applied to organic liquids.\* Crystallization of pipernol occurred preferentially about one electrode after being held molten with a potential of 5000 V between the electrodes. The effect was reversed when the electrodes were interchanged and was interpreted as evidence that the ultra-nuclei were foreign bodies.

**Mr. E. O. Hall** (*Cambridge*) said : The adherence of silver halides to glass, noted in the paper of Zeffoss, Johnson and Egli, is, of course, widely known. However, at the Cavendish Laboratory we have grown single crystals of the silver halides in rod form by the method of Andrade and Roscoe † using Pyrex tubes coated with a thin film of silicone grease. The rods are cast in these coated tubes, from the melt, and from the resulting polycrystalline rods single crystals may be grown in exactly the same manner as metal crystals, although, of course, coated tubes must again be used.

**Mr. H. E. E. Powers** (*London*) said : The phenomenon of the luminosity caused by crushing of sucrose is well known under the name of triboluminescence and can easily be demonstrated by crushing sucrose crystals between sheets of plate-glass in the dark.

It is generally considered to be due to electrical effects and these may be part of the cause of some of our caking phenomena.

Other speakers have spoken of the symmetry of "fault intrusions" into crystals. Many years ago I carried out some work on the production of large candy crystals coloured with caramel. In the course of this work a very large number of crystals of one to two inches in length was examined and in very many cases the coloration though geometric was far from symmetric.

May I, in conclusion, say that in our industry we have a wealth of interesting material and problems, and I should welcome contact and collaboration with any who feel interested in sucrose.

**Dr. K. G. Denbigh** (*Cambridge*) said : In reply to Mr. Powers, I agree that symmetrical inclusions were somewhat rare. The trapping of mother liquor in the angles of a dendritic structure was probably not the only mechanism of the formation of inclusions. I have obtained some evidence that an alternative mechanism depended on the deposition of a speck of solid impurity on the surface of the growing crystal. Fresh crystalline material could not deposit directly on this impurity and the face therefore moved outwards with a fairly wide radius of curvature, creating a pocket of mother liquor with the impurity at the bottom. Crystals were sometimes seen in which there were cavities which had not completely sealed across.

**Dr. A. F. Wells** (*I.C.I., Dyestuffs*) said : In his earlier remarks Dr. Denbigh drew attention to the symmetrical shape of an ice crystal growing on still water. I would suggest that it would be more remarkable if the development were *not* symmetrical. The growing crystal possesses certain symmetry, and it would be expected that the environment of the crystal (in this case, the water) would develop the same symmetry as regards diffusion currents, etc. In the absence of disturbances, therefore, a symmetrical development will occur.

With regard to the experiments of Bunn, Berg and Humphreys-Owen, it would seem dangerous to assume that the phenomena associated with the thick layers observed on crystals growing in supersaturated solution are closely related to the mechanism of slow growth. Many abnormalities are observed in rapid growth from highly supersaturated solutions, particularly the development of faces which do not appear on crystals grown slowly and continuously. The frequent occurrence of inclusions in crystals grown rapidly and the observation that a crystal with obvious internal imperfections often grows more rapidly in the same solution than a clear crystal suggest that the process of desolvation may become an important factor in rapid growth. Before a sodium ion becomes

\* Hammer, *Ann. Physik*, 1938 (5), **33**, 445.

† Andrade and Roscoe, *Proc. Physic. Soc.*, 1937, **49**, 152.

incorporated in the surface of a sodium chlorate crystal it must be half-dehydrated, but before the next layer can be deposited the remaining water molecules must be removed. Thus the spreading of the thick layers observed in some of Bunn's experiments might not correspond to the actual process of incorporation of ions in the crystal direct from the solution but, for example, to some secondary process of ordering in a disordered surface layer containing partially desolvated ions.

**Prof. A. R. Ubbelohde** (*Belfast*) said: I would like to ask Sir John Lennard-Jones how far the contraction he calculated at the surface of ionic crystals would be modified when the crystals are dipped in a medium of high dielectric constant. Under certain circumstances it seems likely that the effect of the high dielectric constant must be to reduce surface strain. In the absence of such reduced surface strain, when a sheet of ions grows outwards over a crystal face there must be a comparatively large discontinuity in the arrangement of ions at the surface region where the uppermost layer terminates, and this discontinuity must travel outwards as the uppermost layer extends.

**Sir John Lennard-Jones** (*Cambridge*) said: I have not calculated the effect on a crystal surface of a highly polarizable medium surrounding it, but it seems clear that for ionic crystals such a medium would produce forces attracting the surface ions outwards. The forces would partly counterbalance the attraction of the rest of the crystal on its surface layer and so tend to eliminate (or reduce) the contraction at the surface.

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### III. ABNORMAL AND MODIFIED CRYSTAL GROWTH

#### Introductory Paper

By A. F. WELLS

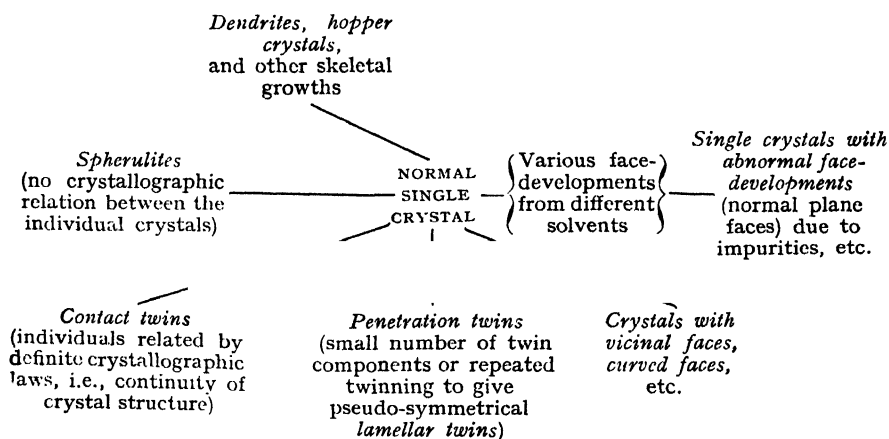
*Received 4th March, 1949*

The discussion of abnormal crystal growth implies that we know what is meant by normal growth, but this is far from being true. A crystal grows by the deposition, layer by layer, of new material on its faces, and the growth on faces of different kinds (i.e., different crystallographic *forms*) is measured as the perpendicular displacement of the face parallel to itself. One object of theoretical treatments of crystal growth is to calculate these rates of growth on the various faces of a crystal in terms of the atomic structure of the crystal and the concentration of material around the crystal. A partial solution of this problem, the calculation of *relative* rates of growth, would answer the purely morphological question: why does a crystal of a particular substance grown under specified conditions develop certain faces? This, however, raises another question: to what extent is the face-development of crystals of a substance constant, external conditions remaining the same? Although it is known that certain face-developments are characteristic of certain crystals, it has never been established experimentally that a crystal with faces of more than one *form* does in fact maintain exactly the same shape during growth, i.e., that the relative rates of deposition on the different faces remain the same. For the present we shall assume that by normal growth is meant the development of a nucleus into a single crystal with plane faces, the relative rates of growth on which are maintained the same throughout growth. We can then classify the various possible types of abnormal growth. Before this is done, however, one other point deserves mention.

All artificial crystals, and most natural ones, are not *ideal* crystals in the sense that a particular atomic arrangement extends without interruption throughout the whole crystal. Instead, the crystal consists of mosaic blocks (within which the structure may be regarded as ideal) which are inclined to one another at small angles. The development of mosaic structure seems such an inevitable feature of crystal growth that it would appear necessary for any theoretical treatment of crystal growth to account for its appearance. (The fact that a few minerals attain, or approach, the ideal state does not necessarily mean that they grew as ideal crystals; they may have been annealed subsequently.) It is known that gross imperfections in internal structure can radically affect the rate of growth of crystals. For example, it is sometimes observed that if two seed crystals, grown in the same way, are grown in the same solution under apparently identical conditions, one may grow very much faster than the other if it has visible internal imperfections. It is tempting to extend this idea of dependence of rate of growth on perfection of internal (and therefore surface) structure, and to suggest that an ideal crystal would not grow at a measurable rate. The numerous anomalies observed in interferometric studies of crystal growth, for example, the cessation of growth on one half of a growing face of a crystal of sodium chlorate while growth proceeds normally on the other half, might then be associated with the perfection of the faces. It may be that if one part of a face accidentally attains an abnormally high degree of perfection, then growth is thereby slowed down. This would appear as reasonable as other explanations, for example, that minute (undetectable) amounts of an unknown impurity settle preferentially on one half of a crystal face. (Alternatively there might be delay in the initiation of an ordering process in a surface layer of randomly oriented, partially solvated, solute.) This complication in experimental studies of crystal growth is one which has not received enough attention, and it may be necessary to ascertain the degree of mosaic structure when comparing growth rates of *different* crystals.

The more important types of abnormal crystal growth are set out below. and I propose to mention briefly some of the problems they raise.

#### TYPES OF ABNORMAL CRYSTAL GROWTH



The account of the morphology of crystals as described, for example, in Groth's *Chemische Kristallographie* is in some cases very misleading, for two main reasons. (1) The face-developments illustrated for many crystals are much more complex than those of crystals grown *slowly* and *continuously*

from pure solutions. They obviously represent, in many cases, crystals which had grown in dishes on laboratory benches and had been subjected to temperature fluctuations leading to alternate partial dissolution and regrowth, and hence to complex face-developments. Crystallographers have always tended to be interested in crystals showing complex face-developments because of the diagnostic value of complex forms, quite apart from the intrinsic beauty of the crystals. (2) Inorganic salts are usually soluble only in water, but many organic compounds are soluble in a variety of solvents, and there is often a crystal habit characteristic of a particular solvent (or set of chemically related solvents). In such cases a single illustration should be replaced by a set of drawings showing these different face-developments.

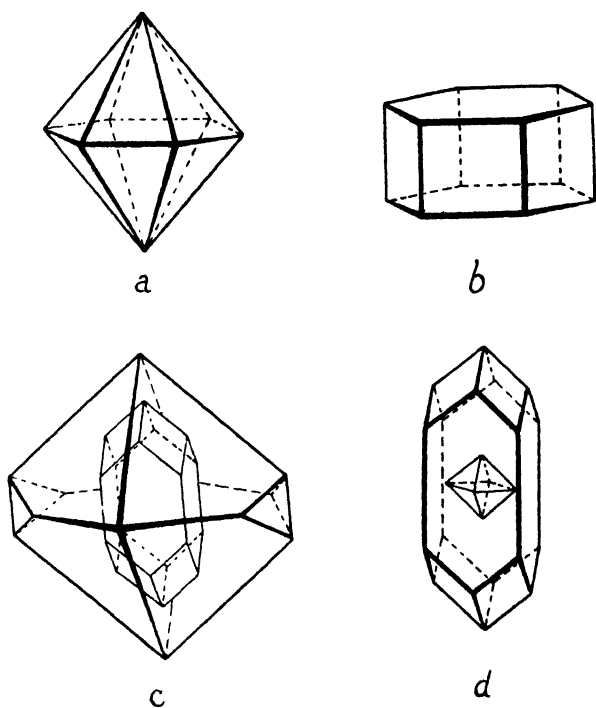


FIG. 1.—Variation of crystal habit with solvent. Above: crystals of iodoform from *a*, aniline, *b*, cyclohexane. Below: crystals of anthranilic acid from *c*, ethyl alcohol, *d*, glacial acetic acid.

Fig. 1 shows examples of crystals which grow with different face-developments from different solvents. Elucidation of habit changes of this type calls for the development of a new branch of surface chemistry involving a study of the interactions of molecules (of solute and solvent) in solution with those in the various crystal faces. Unfortunately, little progress towards even qualitative explanations can be made until the crystal structures of the solutes are known. An exception is provided by resorcinol (*m*-dihydroxy-benzene), which shows some interesting differences in behaviour when grown from different solvents, differences which can to some extent be related to its crystal structure. In the polar crystal of resorcinol (low-temperature form) all the molecules are similarly oriented with respect to the



*c* axis, as shown in Fig. 2, which shows in projection the surface structure of a crystal of the type illustrated in Fig. 3 *a*. The inclusions in such a crystal show that no deposition has taken place on the lower end of the crystal. This is presumably due to the strong interaction of this hydroxylic face with water molecules. If such a crystal is transferred to benzene solution, growth takes place on both ends of the crystal (Fig. 3 *b*). In this solution there is no preferential interaction between solvent molecules and a hydroxylic as compared with a benzenoid face. Unidirectional growth also takes place in certain other solvents, and from ethyl acetate a remarkable shape develops (Fig. 3 *c*). This is a conical crystal terminated by two normal plane ( $0\bar{1}\bar{1}$ ) and ( $01\bar{1}$ ) faces, and growth takes place only on these faces. No deposition occurs on the lower (conical) end of the crystal. No explanation has yet been found for this extraordinary crystal shape, which is the normal development from ethyl acetate solution.

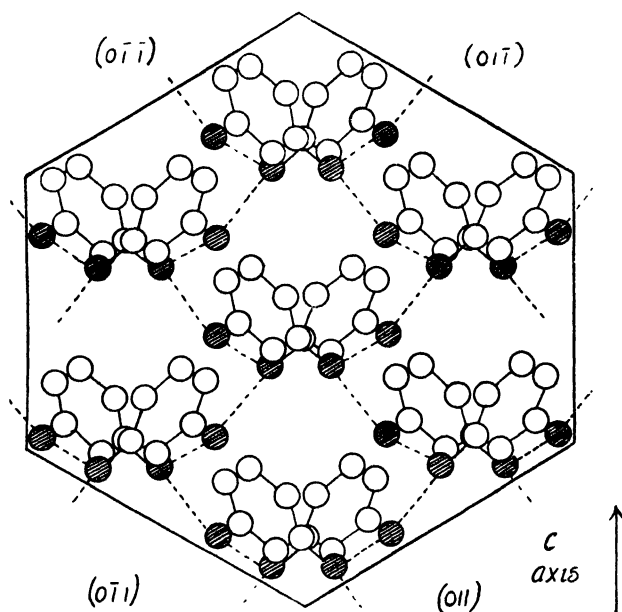


FIG. 2.—Projection of the structure of resorcinol on (100) showing the surface structure of ( $01\bar{1}$ ) and ( $01\bar{1}$ ) faces. The shaded circles represent OH groups and the broken lines, O-H-O bonds.

Closely related to the effect on crystal habit of change of solvent is the effect of impurities in solution. Preferential interaction between the atoms or groups in certain crystal faces with either solvent or impurity alters the relative rates of growth on faces of different types, resulting in change of habit. Much of the experimental work on the effect of adsorbed impurities has been carried out with complex dyes and, as might be expected, it is difficult to account for the remarkably specific action of many of these complex molecules in terms of the structures of the adsorbed molecule and of the crystal surfaces. This is emphasized in the papers of Buckley, and of Butchart and Whetstone, which follow. An interesting application of this kind of habit change is described by Whetstone, who has found that caking of certain soluble salts is due to the formation of intergranular bridges, the mechanical strength of which can be considerably reduced by modifying the habit of the recrystallized material formed between the granules.



*a*



*b*



*c*

FIG. 3. - Crystals of resorcinol (low-temperature form) : *a*, crystal from water, showing inclusions; *b*, crystal of type *a*, grown larger in benzene solution; *c*, crystal from ethyl acetate.



The unidirectional growth of resorcinol in water and some other solvents shows in a striking way the importance of interaction between molecules in the surface of a crystal and solvent molecules. It suggests that even in cases where this interaction is less powerful, desolvation of the solute molecules may be an important factor to be considered in the growth of crystals from solution. Even when a solute molecule (or ion) has settled on a crystal face it has been only half-desolvated, and before the next layer can be laid down the remaining solvent must be removed. Under certain conditions, particularly during rapid growth, all this solvent is not removed and inclusions are formed in the crystal. In a similar way, adsorbed molecules may be trapped in the growing crystal, as in the case of coloured crystals of inorganic salts mentioned by Buckley. In some cases included molecules or crystallites are oriented in the host-crystal and give rise to pleochroism.

A phenomenon closely allied to the deposition of oriented crystallites on the surface of a *growing* crystal is the formation of oriented overgrowths on crystal faces. The literature of this field is very extensive, and much of the interpretation of the experimental facts has been concerned primarily with the geometrical aspect, i.e., the fitting of the overgrowth to the substrate. Two papers in this section deal with the energetics of the formation of oriented overgrowths. Rhodin deals with thin aluminium films deposited on the surfaces of inorganic crystals, mostly ionic in character, and van der Merwe has made a valuable survey of the literature in connection with a theoretical study of the conditions which must be satisfied for the formation of an oriented, crystalline, overgrowth. A third paper in this field, by Hocart, describes observations on oriented overgrowths of ammonium nitrate on mica made in connection with a study of the stabilization of the high-temperature forms of the salt by incorporation of small amounts of other salts with suitable lattice-constants.

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## MISFITTING MONOLAYERS AND ORIENTED OVERGROWTH

BY J. H. VAN DER MERWE

*Received 3rd February, 1949*

Crystal orientations are not in general determined by long-range forces, but by forces between one atomic layer and the next. Hence, in order that there shall be a definite orientation in a crystalline overgrowth on a crystalline substrate, there must be formed, as the initial stage, an immobile monolayer of regular atomic pattern, to be called an "embryo." If the formation of a monolayer is regarded as a process of adding atom to atom, it is possible, if the influence of the substrate is strong, for these (foreign) atoms to take up the same positions on the substrate as would atoms belonging to the same substance as the substrate. The resulting monolayer is therefore homogeneously deformed to fit on the substrate, thus forming an embryo. "Oriented overgrowth" is then obtained when the atomic pattern (unchanged, when the final overgrowth is pseudomorphic, or homogeneously deformed, when the abnormal strain is released, at some

stage, by lateral expansion or contraction) and orientation of the embryo are preserved throughout the entire lattice of the overgrowth.

A theory has been developed<sup>1</sup> which led to predictions regarding the necessary conditions under which an embryo can be formed. The theory is based on the properties of a one-dimensional dislocation model, consisting of a row of identical balls, connected by identical springs (force constant  $\mu$ ); the balls at the same time being acted on by a force, which varies periodically with the position on the substrate. The first harmonic term (amplitude  $\frac{1}{2}W$ ) in a Fourier series is taken to represent the corresponding potential energy. There may be a difference between the natural spacing  $b$  of the balls and the wavelength  $a$  of the substrate field. In the application to embryo formation the configuration of balls and springs is taken to represent the monolayer, and the periodic force to represent the substrate's influence on the deposit atoms; this extension from one to two dimensions can be shown to be justified.

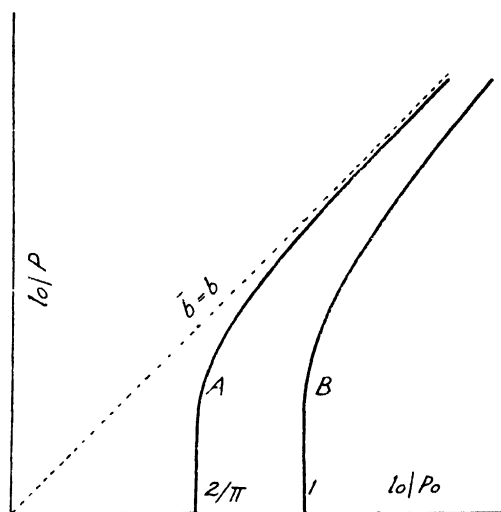


FIG. 1.—Graph of  $l_0/P = l_0(b/a - 1)$  against  $l_0/P_0 = l_0(b/a - 1)$ . A. Lowest energy state. (N.B.  $l_0/P = 0$  for  $0 \leq l_0/P_0 \leq 2/\pi$ .) B. Spontaneous generation of dislocations.

It is found that with this model the fit or misfit of the monolayers and substrate is naturally described in terms of "dislocations." Thus, if there is misfit so that 99 or 101 atoms in a row lie over 100 potential troughs, the majority of the atoms actually lie nearly at the bottom of their troughs, while there is a small region where the atoms ride over the crests, to miss a trough or squeeze an extra atom in. This region of misfit we call a surface dislocation: if a perfect crystal is built above it, it will develop into an ordinary crystal dislocation of the kind originally proposed by Orowan and Taylor to account for the mechanical properties of solids. The mathematical theory of our model shows that when the natural spacing  $b$  differs from that,  $a$ , of the substrate, the lowest energy state of the system remains one with *no dislocations* up to a certain critical value of the misfit  $1/P_0$  defined by

$$1/P_0 = (b/a - 1)_{\text{critical}} = 2/\pi l_0,$$

where

$$l_0 = (\mu a^2/2W)^{\frac{1}{2}}.$$

Calculation with Lennard-Jones forces, assuming the interactions with

<sup>1</sup> Frank and van der Merwe, *Proc. Roy. Soc. A* (in press).

other atoms of the deposit and with the substrate atoms are similar, shows that  $l_0$  is about 7. Thus the critical misfit should be about 9 % in an average case. There will, however, in general be a large variation about this average value, depending on the relative forces exerted by deposit atoms on each other (giving  $\mu$ ) and on the substrate (giving  $W$ ), respectively. This is not the only critical condition of importance, for there still remains an activation energy for the generation of dislocations,\* which only falls to zero at a larger degree of misfit  $1/l_0$  (equal to 14 % in the average case). Hence, below this critical misfit, it is also possible at low temperatures for the monolayer to be deposited in fit with the substrate, thus producing an embryo in a metastable state. Fig. 1 shows that the density of dislocations  $\bar{b}/a - 1$ , where  $\bar{b}$  is the average spacing of deposit atoms, rises abruptly to a large value on passing either of the critical misfit conditions, the lower of which is probably significant for high, and the higher for low, temperatures. Once there is a high density of dislocations (at which incidentally the spacing of the deposit layer becomes

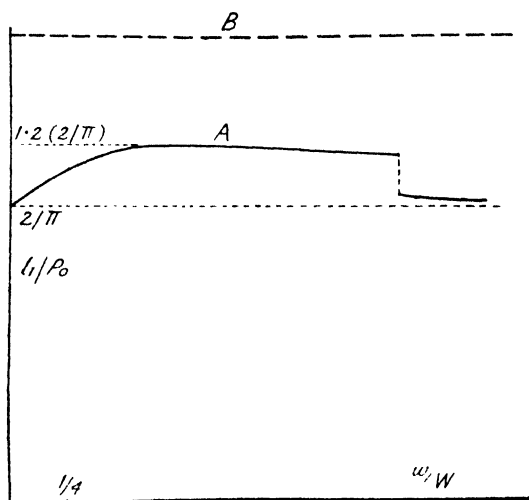


FIG. 2.—Graph of  $l_1/P_0$  against  $w/W$ . A. Lowest energy state. B. Spontaneous generation of dislocations.

practically equal to its natural spacing and independent of that of the substrate) the monolayer should be quite mobile on the surface, free to rotate as well as to glide. Such a monolayer cannot be an embryo for fully oriented overgrowth, though, of course, there may be a preferred axis normal to the surface, as also occurs on amorphous substrates.

It is unlikely that the variation of potential energy in an actual case will be represented accurately by a single sinusoidal term. The corresponding curve is expected to have in general a maximum which is flatter and wider than its minimum. This can be attained by introducing into the potential representation a second harmonic term of small amplitude  $\frac{1}{2}w$ . Increasing  $w/W$  beyond  $1/4$  makes the potential curve change its nature; it develops a second minimum. Thus the introduction of a second harmonic term was found to be convenient in investigating the influence of the shape of the potential curve on the critical properties of the system. The outcome of the investigation<sup>1</sup> is represented graphically in Fig. 2. It is seen that the limiting misfit corresponding to spontaneous generation of complete disloca-

\* This spontaneous generation is only possible at the edge of the layer, and, of course, becomes impossible when any flat region of the surface is completely covered.

tions (displacement vector  $\vec{d}$ ) does not depend at all on the actual shape of the potential curve, but only on its maximum variation  $W_0$ , according to the formula  $1/P_0 = 1/l_1$ , where  $l_1 = (\mu a^2/2W_0)^{1/2}$ . This result was shown to be completely general, holding for any shape of periodic potential curve of wavelength  $a$ . The effect on the critical misfit corresponding to the state of lowest energy of the system is to increase this misfit; the increase having a maximum value of approximately 1.2 times the original value at  $w = 0$ . This corresponds to a shift of the critical value of 9 % to approximately 11 % (assuming  $W_0$  to remain constant). We may therefore conclude that the actual shape of the potential curve is of secondary importance in embryo formation, and that it is its maximum variation  $W_0$  which is the important factor. Note that  $W$  always occurs in the ratio  $W_0/\mu$ . We shall come back to the significance of  $W_0$  and  $W_0/\mu$  when we discuss the experimental evidence.

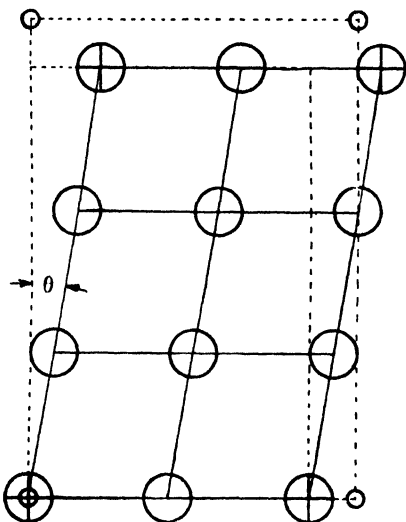


FIG. 3.

- O denotes troughs of potential field.
- + denotes suitable troughs for fitting of deposit units.
- o denotes natural positions of deposit units.
- $\theta$  = angle through which the monolayer must be sheared to fit on the substrate.

Having described the conditions necessary for the formation of an embryo, the next step is to explain how an oriented deposit can grow from it. This embryo is a suitable substrate for the formation of another embryo on it, provided the binding between deposit atoms is not weaker than their binding on to the substrate. If the atomic pattern of the embryo, and hence that of the substrate, resembles the atomic pattern of a plane in the normal lattice of the deposit (e.g., when the two lattices are isomorphic), it is possible for a stable, macroscopically thick, oriented film to grow by repetition of this process of embryo formation. This assumes, of course, that any flat region of the surface is *completely* covered by the first monolayer before the second layer is appreciable (see below).

Since the formation of a monolayer is really a process of adsorption on the substrate, it is the pattern of potential troughs, i.e., positions of minimum potential energy, of the deposit atoms in the substrate field, rather than the atomic pattern of the substrate surface, which must resemble the atomic pattern in a plane of the deposit structure. To realize the need of this distinction it is only necessary to consider the case of a neutral argon atom

on the (001) face of NaCl, for which the potential troughs are at the centres of the small squares having alternately  $\text{Na}^+$  ions and  $\text{Cl}^-$  ions at their corners,<sup>2</sup> as compared with the case of a  $\text{K}^+$  ion on the same substrate for which the potential troughs are presumably on top of  $\text{Cl}^-$  ions. This example also shows that, for the sake of generality, it is convenient to speak of deposit or substrate "units," since these can be atoms, molecules, ions, etc. An illustration of a more general case of fitting deposition units in substrate potential troughs is shown in Fig. 3.

Note therefore that a difference in the shapes of corresponding patterns, e.g., a shear as in the Fig. 3, also represents "misfit," measured by  $\tan \theta$ . This case is in fact covered by the theory.<sup>1</sup> The orientation of alkali halides on  $\text{NaNO}_3$  with  $\tan \theta = 0.21$  is an example.

The thickening of films will, however, certainly cause the generation of dislocations at free boundaries of an initially undislocated film, since the energy to compress the thick film will be much greater. For example, a double layer will have a critical misfit of the order of  $(2)^{-1}$  times that of a monolayer (taking  $\mu$  for a double layer to be twice that for a monolayer). However, once the embryo covers the whole flat area, it no longer has "free boundaries on a flat substrate," since it will also be completed around corners and edges, thus pinning the boundaries to the substrate. It is therefore possible for a stable oriented film to grow pseudomorphically with the substrate.

Even if, during the early stages of growth, spontaneous generation of dislocations does take place at free boundaries in planes parallel to the substrate, the initial orientation will be preserved in subsequent layers provided the dislocated layer is at least a few (say, of the order of four) monolayers thick, for the irregularities in the atomic pattern existing at the centres of dislocations will be largely smoothed out over this thickness. We know, on the other hand (if spontaneous dislocation does not take place during the early stages of growth), that the large strain, permissible in thin layers, cannot persist into films of indefinite thickness; a fact also well established in experiments showing that pseudomorphic growth was no longer observed in sufficiently thick films.<sup>3b</sup> It will be impossible to grow macroscopically thick films with more than, say, 0.1 % of strain, corresponding to the yield stress of the bulk material. Hence thickening of films must necessarily be accompanied by transition processes which make the bulk of thick deposits strain free.

These theoretical ideas are in good general agreement with experimental observations. The fact that pseudomorphic overgrowth is observed seems to show that there are cases in which slip does not take place during the early stages of growth. Amongst the most striking examples are the cases of Al on Pt<sup>3b</sup> and ZnO on Zn,<sup>3a,b</sup> Similar tendencies were observed in overgrowths of MgO on Mg,<sup>8b</sup> Ni and Co on Cu,<sup>4c</sup> and in the experiments of Finch and Sun,<sup>5</sup> where the abnormal crystal orientations of very thin films were in general such that the atomic population density in the orientation plane of the deposit approached that in the substrate surface. More experimental observations on very thin films would be very useful.

In all cases, whatever the mechanism of the slip process, some residual stresses are likely to remain. There is plenty of experimental evidence for this<sup>5,6</sup> from the behaviour of stripped films, though one must always consider the possibility of strains caused by the stripping process.

<sup>2</sup> Orr, *Trans. Faraday Soc.*, 1939, **35**, 1247.

<sup>3</sup> Finch and Quarrell, (a) *Proc. Physic. Soc.*, 1934, **46**, 148; (b) *Proc. Roy. Soc. A*, 1939, **141**, 398; (c) *Trans. Faraday Soc.*, 1935, **31**, 1051.

<sup>4</sup> Menzer, (a) *Naturwiss.*, 1938, **26**, 385; (b) *Z. Krist.*, 1938, **99**, 378; (c) 1938, **99**, 410.

<sup>5</sup> Finch and Sun, *Trans. Faraday Soc.*, 1936, **32**, 852.

<sup>6</sup> Goche and Wilman, *Proc. Physic. Soc.*, 1939, **51**, 625.



The most important effect of the strain transition process is the possibility of a loss or change in the initial orientation. If this process takes place through slip in planes parallel to the substrate, as we assume is the case in experiments<sup>7,8</sup> where the orientations of the small deposit crystals are determined under the microscope, such a loss is not very likely. If, however, the slip takes place simultaneously in planes inclined to each other, a loss is likely to occur. This was presumably the case in the experiments of Finch and Sun<sup>5</sup>; the initial regular orientation became almost random with sufficient film thickness. The transition process can, however, also take place through the growth of an unstrained bulk film on the thin strained part of the overgrowth at the contact surface. Detailed calculations by Menzer<sup>4</sup> (confirmed by Goche and Wilman<sup>6</sup> in the case of Ag) on observations of Ag and Ni films on NaCl<sup>9</sup> showed that the bases of the deposits consisted of small crystallites (in four orientations rotated through 90°) having (221) faces in contact with the substrates. The bulk of the film, growing on these crystallites, is twinned on the (111) faces with respect to the crystallites and has an orientation parallel to that of NaCl. The corresponding misfits (9 % for Ag, 7 % for Ni) in the contact plane thus also lie within the tolerance limit, which is not the case for the misfits (−27 % for Ag, −38 % for Ni) suggested by the orientation of the bulk of the overgrowths. Many of the orientations of metallic overgrowths on ionic crystals are likely to belong to similar types, and are therefore given in a separate table (Table II). These orientations are in general such that better fit can be achieved by other orientations, as was shown by Thomson<sup>10</sup> for some cases. These considerations, together with the fact that deposits on a random substrate have a tendency to expose a definite plane, show that one cannot be certain that the orientation of an overgrowth is the same as that of the initial embryo.

If we assume that strong adsorption of the overgrowth on to the substrate can in general be expressed by a large  $W_0$ , then it is in agreement with the theory that strong adsorption is an essential condition for preferred orientation, as has been established by various workers<sup>7,8,11,12,13,14,15</sup> as a result of experiments on "partners" (combination of deposit and substrate) which yielded no oriented overgrowth in spite of ideal geometrical conditions. The binding in the adsorption processes was of various types, e.g., through a hydrogen bond,<sup>8i</sup> through dipoles,<sup>7g</sup> etc. Willems, having drawn the general conclusion that, for oriented overgrowth to take place, there must exist the possibility of a strong chemical bond between the units of the overgrowth and the corresponding units of the substrate, confirmed it experimentally.

<sup>7</sup> Neuhaus, *Z. Krist. A*, (a) 1941, **103**, 297; (b) 1943, **105**, 187; *Naturwiss.*, (c) 1943, **31**, 33; (d) 1943, **31**, 387; (e) 1944, **32**, 34; (f) 1948, **35**, 27; (g) *Z. physik. Chem. A*, 1943, **191**, 359; (h) 1943, **192**, 309; (i) *Neus. Jb. Miner., Geol., Paläont.*, 1943, **78**, 189; (j) *Z. Elektrochem.*, 1944 (in press).

<sup>8</sup> Willems, *Z. Krist. A*, (a) 1938, **100**, 272; (b) 1943, **105**, 53; (c) 1943, **105**, 144; (d) 1943, **105**, 149; (e) 1943, **105**, 155; *Naturwiss.*, (f) 1941, **29**, 319; (g) 1943, **31**, 146; (h) 1943, **31**, 208; (i) 1943, **31**, 232; (j) 1943, **31**, 301; (k) 1944, **32**, 324; (l) *Ber.*, 1943 (in press); (m) *Kolloid-Z.*, 1940, **90**, 298.

<sup>9</sup> Brück, *Ann. Physik*, 1936, **26** (5), 233.

<sup>10</sup> Thomson, *Proc. Physic Soc.*, 1948, **61**, 403.

<sup>11</sup> Sloat and Menzies, *J. Physic. Chem.*, 1931, **35**, 2005.

<sup>12</sup> Seifert, *Fortsch. Miner.*, (a) 1935, **19**, 103; (b) 1936, **20**, 324; (c) 1937, **22**, 185; *Z. Krist. A*, (d) 1937, **96**, 111; (e) 1938, **99**, 16; (f) 1939, **100**, 120; (g) 1940, **102**, 183.

<sup>13</sup> Heintze, *Z. Krist.*, 1937, **97**, 241.

<sup>14</sup> Royer, *Bull. Soc. franc. Miner.*, (a) 1928, **51**, 7; *Compt. rend.*, (b) 1925, **180**, 2050; (c) 1932, **194**, 620; (d) 1932, **194**, 1088; (e) 1933, **196**, 282; (f) 1933, **196**, 552; (g) 1937, **205**, 1418.

<sup>15</sup> Vineyard, *Physic. Rev.*, 1942, **61**, 100.

Amongst the most interesting experiments for the present theory, from the point of view of binding, are those on ionic partners. These experiments show that the limiting misfits, in cases where both partners are ionic, are much greater than when one of the partners is not ionic. This indicates that the electrostatic forces are the important binding components in these cases. These experiments provide special opportunities to test conclusions from the present theory, if we make the following assumptions—

(i) The electrostatic binding is the important factor in the adsorption energy.

(ii) Stronger adsorption, and hence larger  $W_o$ , can be attained by (a) using solvents (from which to deposit overgrowth) of lower dielectric constant and (b) closer approach of deposit units to the substrate, which will be the case if the ionic radii of the deposit units and/or those of the substrate, are small. Hence preferred orientation for partners, having misfits in the region of the tolerance limit, will be sensitive to small variations in  $W_o$ , i.e., partners which do not orientate under certain conditions will do so under conditions for which  $W_o$  is greater. Thus Willems,<sup>8</sup> Sloat and Menzies<sup>11</sup> established that the tolerance limit could be increased by using solvents of lower dielectric constants. In the case of Sloat and Menzies, this was as much as 7 %. They also showed that NaCl had an appreciably greater orientating ability than KCl. This is to be expected since the ionic radii are 0.98 Å for  $\text{Na}^+$  and 1.33 Å for  $\text{K}^+$ , thus making  $W_o$  (for NaCl) greater than  $W_o$  (for KCl).

In the preceding we have assumed that the properties of the original simple model (identical balls, i.e., a single  $W_o$ ) also apply for compound overgrowths (non-identical balls). Only the fact that the deposition units (ions in a special case) are of different size is sufficient justification for the use of  $W_o = W_1$ , for the one unit, and  $W_o = W_2$ , for the other unit, where  $W_1 \neq W_2$ . This problem has been solved<sup>16</sup> by using parabolic arcs in the potential representation, since it could not be solved for a Fourier representation. The resulting expressions show that the corresponding limiting misfits increase with  $(W_1 + W_2)/\mu$ .

It is also in agreement with the theory that the limiting misfits in the case of ionic overgrowths should be greater than that for metallic overgrowths (assuming the adsorption is not much different), since the compressibilities of the former are much greater than those of the latter. In particular, oriented overgrowth with very large misfits is observed in the case of oxides and iodides—a fact which we can connect with the particularly high compressibilities of these large anions.

The general problem of oriented overgrowth of a non-isomorphic deposit on various surfaces of a substrate is exceedingly complicated, but from similar theoretical considerations as those above one may anticipate that a preferred orientation can exist when there is a similarity in spacing in one row of closely packed units in each lattice, as concluded by various workers.<sup>6, 13, 14</sup> Seifert,<sup>12</sup> in his work on oriented overgrowth of ionic partners, came to the conclusion that one-dimensional lattice fitting for a row of closely spaced ions of alternating sign is sufficient to give rise to oriented overgrowth.

I am indebted to Dr. F. C. Frank and Prof. N. F. Mott for their keen interest in this work and their many valuable suggestions. I also have to thank the South African Council for Scientific and Industrial Research for a grant and special leave, which rendered it possible to perform this research.

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<sup>16</sup> van der Merwe (to be published elsewhere).

## Tables

In Tables I to III—

*Column 1* gives the substrate material, the exposed crystal face and a reference axis lying in this face.

*Column 2* gives the material deposited, the crystal face in contact with the substrate, and the crystal axis parallel to the reference axis in column 1. Where the face and/or axis in either column 1 or 2 is missing it is supposed to be that just above it.

*Column 3* gives the percentage excess of the lattice spacing of the deposit, relative to the substrate; in the case of non-isomorphic relationship, the misfits in two orthogonal directions are given.

*Column 4* gives the literature by number, and remarks by numbers with asterisks.

The *underlined entries* give a few of the numerous cases in which oriented overgrowth was not observed under conditions closely comparable with the preceding analogous cases. The misfit is calculated in these cases for the orientation given. No attempt has been made to give an exhaustive list of negative cases.

TABLE I

CASES IN MOST OF WHICH NORMAL ORIENTED OVERGROWTH IS BELIEVED TO OCCUR

Substrate		Deposit		Misfit %	Literature + Remarks
Pt	(111) [110]	Al*	(111) [110]	3	3 b; 1*
		Au		4	3 c
<u>Pt</u>		Zn		-4	3 b
		Mg		16	3 b
Cu	(110) [001]	Cu	(110) [001]	-8	5
		Co		10	5
		Ni		11	5
		Cr		2	18
		Co*		-1	18; 2*
<u>Cu</u>	(111) [110]	Ni	(111) [110]	-3	18; 3*, 2*
		Ag		13	18
		Zn		4	18
		Cd		16	18
		Au	(110) [110]	13	5; 4*
Au	(111)	Fe		12, -9	5
	(100) [011]		(100) [010]	0	5
	(111) [110]		(110) [001]	0, 18	5
	(100) [010]	Co	(100) [010]	-13	5
	(111) [110]†		(111) [110]	-14	5
	(100) [011]	Ni	(100) [010]	24	5
	(111) [110]†			6, 24	5; 5*
		Ag	(111) [110]	0	5, 9
		Pt		-4	5
		Cu		-13	5
Pd	(100) [001]†		(100) [001]	-7	5
	[011]	Fe		4	5
	(110) [110]	Au	(110) [110]	12	5
		Cu		3	5
		Fe	(100) [001]	15, -19	5
Ag	(001) [100]	Au	(001) [100]	0	9
		Cu <sub>2</sub> O		18	9, 19 a, 20, 21, 22, 23, 24; 6*, 2*
α-Fe		FeO	[110]	6	19 a, b
Mg		MgO	(111) [110]	-7	3 b; 2*
Pd		PdO	(001) [100]	11	24

[Cont.]

TABLE I (Continued)

Substrate	Deposit	Misfit %	Literature + Remarks
Zn	(0001) [1010]	20	3 a, b; 1*
Ag	(001)† [100]†	12	25
Ag	(001) [110]	0	17, 25
	AgBr	—4	17
	AgCl	13	17
	AgI	6	17
Cu			
Ag	[100]	0, —14	17
	AgBr	—4, —17	17
	AgCl	—3	22
Cu	[110]	—9	22
	(111)	12	22; 2*, 7*
Ag	(001) [010]	—3	6, 9
	[100]	1	11
Ag		—1	11
NaCl	(001) [100]	5	11
	NH <sub>4</sub> Cl	6	11, 14
	NaBr	6	11, 14
	NaCN	15	11, 14
	NaI	—5	11, 14
	KF	12	11, 14
	KCl	16	11, 14
	KCN	17	11
	KBr	25	11
	KI	—9	11, 14
	LiCl	—3	11, 14
	LiBr	1	11, 14
	AgCl	3	14, 14
	AgBr	3	14
	AgCN	30	14, 14
	RbI	16	14
	NH <sub>4</sub> Cl	23	14
	NH <sub>4</sub> Br	29	14
	NH <sub>4</sub> I	6	14
	PbS	—18	14
NaCl	NaF	17	14
	RbCl	4	14, 14 a
KCl	KCN	5	14, 14 a
	KBr	12	14
	KI	—10	11, 14 a
	NaCl	—5	11, 14 a
	NaBr	—5	11, 14 a
	NaCN	3	11, 14 a
	NaI	—8	11, 14 a
	AgCN	—12	11
	AgCl	—8	11, 14 a
	AgBr	—13	11, 14 a
	LiBr	4	11
	NH <sub>4</sub> Cl	10	11
	NH <sub>4</sub> Br	15	11
	NH <sub>4</sub> I	5	11, 14 a
	RbCl	9	11, 14 a
	RbBr	17	11
	RbI	5	11
	PbS	—15	11
KCl	KF	4	26 d
TiCl	TiBr	6	26 d
TiBr	AgBr	4	26 b
AgCl	AgCl	—2	26 d
TiCl	AgBr	3	26 d
TiBr	TiI	10	26 d
TiCl	AgCl	—4	26 d

[Cont.]

TABLE I (Continued)

Substrate		Deposit		Misfit %	Literature + Remarks
AgBr	(111) [110]	AgI	(001) [100]	13	26 c
PbS	(001) [100]	NaCl		-6	11, 14 a
		NaBr		-1	11, 14 a
		NaCN		-1	11, 14 a
		NaI		8	11, 14 a
		KCl		5	11, 14 a
		KCN		10	11, 14 a
		KBr		10	11, 14 a
		KI		18	11
		AgCl		-7	11, 14 a
		AgBr		-4	11, 14 a
		RbCl		10	11, 14 a
		RbBr		15	11, 14 a
<u>PbS</u>		LiBr		-8	11
		<u>NH<sub>4</sub>I</u>		21	11
MgO	(001)† [100]†	NaF	(001)† [100]†	10	15
		LiF		-5	15
<u>MgO</u>		NaCl		10	15
FeO	(001) [100]	Fe <sub>3</sub> O <sub>4</sub>	(001) [100]	-3	19 b
Fe <sub>3</sub> O <sub>4</sub>		FeO		3	19 b
ZnS	(110) [110]	ZnO	(1013) [0100]	16	12 g; 8*
α-Al <sub>2</sub> O <sub>3</sub>	(1120) [0001]	NiO	(111) [112]	-7*	27; 9*
	(1011) [r-ε*]		(110) [110]	0, 16*	27; 10*
CaCO <sub>3</sub>	(100) [010]	NaNO <sub>3</sub>	(100) [010]	1	28
		NaCl		-12, t* = 21	14 f, 11*
		NaBr		-7	14 f
		NaI		1	14 f
		KCl		-2	14 f
		KBr		3	14 f
		KI		10	14 f
		RbCl		3	14 f
		RbBr		7	14 f
<u>CaCO<sub>3</sub></u>		RbI		14, t = 21	14 f
<u>NaNO<sub>3</sub></u>		NaCl		-13, t = 23	13, 14 f; 11*
		NaBr		-8, t = 23	13, 14 f
		NaI		0, t = 23	13, 14 f
		KCl		-3, t = 23	13, 14 f
		KBr		2, t = 23	13, 14 f
		KI		9, t = 23	13, 14 f
		LiCl		-21, t = 23	13
		LiBr		-15	13
		KMnO <sub>3</sub>		-3	31
KClO <sub>3</sub>		Urea	(001) [110]	4	29
NH <sub>4</sub> Cl	(001) [100]			1	29
NH <sub>4</sub> Br				10, -17	29
KCl	(111) [110]		(111) [110]	1, -7	29
NaCl			(110)	1, 15	29
	(001) [100]		(111) [110]	14, 21	29
<u>KBr</u>	(111) [110]	<u>Urea</u>			
		Thiourea	(010) [001]	12, 2	7 f
ZnS	(110) [110]		(101) [010]	0, -6	7 f
			(001) [100]	1, 0	14 d
PbS	(001) [110]		(100) [001]	3, -8	7 f
NaCl	(001) [110]	A*	(010) [001]	-1, 23	8 f; 12*
NaNO <sub>3</sub>	(100) [011]	β-H*	(1010) [0001]	10, 11	8 b; 12*
		α-H		7, -2	14 d; 12*
		β-H		10, 13	8 b
		α-H		11, 1	14 d
Siderite				2, 19	8 b
Rhodochrosite				2, 19	8 b
Zincspar				0, 21	8 b
Magnesite				2, 21	8 b

[Cont]

TABLE I (Continued)

Substrate	Deposit	Misfit %	Literature + Remarks
Dolomite (100) [011]	$\alpha$ -H (1010) [0001]	4, 16	8 b
Baryte (001)		3, 3	8 e
Celestite (100)		3, -12	8 e
(001)		6, 7	8 e
*CaCO <sub>3</sub> (100) [dl]	$d$ -G (b-c) [c-axis]	6, -16	8 e
*Siderite	S-a	-8, 0	8 b; 13*
Urea [110]	NH <sub>4</sub> Cl (001) [100]	0, 4	8 b; 13*
Mica (Muscovite) [100]	NH <sub>4</sub> Br (001) [100]	-4	29
	NaI (111) [110]	-1	29
	KCl	-12	30
	KBr	-14	14
	KI	-11	11, 30
	RbCl	-4	14, 30
	RbBr	-10	14
	RbI	-6	14
	KMnO <sub>4</sub> (001) [010]	0	14, 30
	KClO <sub>4</sub>	11, 2	14 b
Chlorite	NH <sub>4</sub> ClO <sub>4</sub>	10, -1	14 b
	KMnO <sub>4</sub>	13, 4	14 b
	KClO <sub>4</sub>	8, 1	14 b
	NH <sub>4</sub> ClO <sub>4</sub>	7, -4	14 b
Mica (Muscovite) [110]	$\alpha$ -H (1120) [0001]	9, 0	14 b
[010]		9, 6	8 e
	Thiourea (010) [001]	8, 7, $t = 0$	8 c
	MgSO <sub>4</sub> ·7H <sub>2</sub> O (100) [001]	-4, 6	7 f
Mica (Muscovite) (001) [120]	MnSO <sub>4</sub> ·7H <sub>2</sub> O	1	14 c; 14*
	NiSO <sub>4</sub> ·7H <sub>2</sub> O	0	14 c; 14*
Gypsum (010) [301]	Urotropins (110) [110]	0	14 c; 14*
CaF <sub>2</sub> (111) [110]	LiCl (110) [110]	1, 8, $t = 14$	8 d; 11*
	LiBr	-6	33
	NaCl	0	33
	NaBr	3	33
	KCl	8	33
	KBr	14	33
		21	33

1\*. Pseudomorphic overgrowth.

2\*. Pseudomorphic tendencies.

3\*. Orientation does not persist in thick films.

4\*. Direction on plane denoted by † is not given by the authors, and thus assumed to be that given.

5\*. All potential troughs (face-centred cubic and hexagonal) are assumed to be occupied by deposit units.

6\*. Cu<sub>2</sub>O has tendency to expose (110) face.7\*. Cu<sub>2</sub>S deposit has cubic symmetry, thus differing from normal structure. (Pseudomorphism?)

8\*. Author explains types of orientations on different faces of ZnS.

9\*. "Fit" oxygen atoms against each other.

10\*. Half of deposit units (when deformed for "fit") are on potential crests.

11\*.  $t = \tan \theta$  (in %), where  $\theta$  = angle through which to shear deposit pattern in order that it might resemble the substrate pattern.Two orientations differing by  $\theta$ .12\*. A  $\equiv$  anthraquinone;  $\alpha$ -H  $\equiv$   $\alpha$ -hydroquinone;  $\beta$ -H  $\equiv$   $\beta$ -hydroquinone.\*13\*.  $d$ -G  $\equiv$   $d$ -glucose; S-a  $\equiv$  salicylic acid; (b-c)  $\equiv$  plane through b- and c-axes; dl  $\equiv$  long face-diagonal.

14\*. Of the deposit units (when "fitted") 1/3 are on potential crests, 1/3 on intermediate positions and 1/3 in troughs.

r-e\*  $\equiv$  rhombohedral edge.

TABLE II

CASES, IN MANY OF WHICH A VARIETY OF ORIENTATIONS OCCUR, IN WHICH IT IS SUSPECTED THAT THE FINAL ORIENTATION IS ESTABLISHED THROUGH INTERMEDIATE LAYERS OF DIFFERENT ORIENTATION, ANALOGOUS TO THE CASES ANALYSED BY MENZER\*

Substrate			Deposit		Misfit %	Literature + Remarks		
NaCl††	(001)	[100]	Ag	(001)	[100]	-27	9; ††1	
		[110]		(111)	[110]	26, -27	9	
		[110]†		(221)*		9, 9**	4, 6; 2*, 3**	
		[100]†				3	4	
		[100]		(001)	[100]	-38 or 24*	9; 4*	
		[110]†		(221)*	[110]	-7, -7**	4; 2*, 3**	
		[100]				-12	4	
		[110]		Au	(111)	[110]	25, -28	9
				Al			24, -28	9
		[100]		Cu	(001)	[100]	-36 or 28*	9; 4*
		[110]		Co			-11	9
		[100]		Pd			-31	9
		[110]					-3	24
				Fe			-28	9
		[100]		(a)			1	34 a, b
				(b)				
				(c)	(110)	[110]	1, -28*	9, 34 a, b; 4*
					(001)	[100]	-28	34 a, b
		(111)	[112]	2, -12*	34 b; 4*			
		(210)	[121]	2, x*	34 b; 6*			
	Cr	(110)	[001]	2, -28	34 c; 9			
		(111)	[112]	2, -12*	34 c; 4*			
		(210)	[001]	-19, -28	34 c			
NaCl††		Cr			2; -28; 2,	34 c; 5*		
					-28			
		Mo			11; -21; 11,	34 d; 5*		
				-21				
KCl††		Fe			-9; 29; -9,	34 a; 5*		
					29			
KBr††					-12; 23;	34 a; 5*		
					-12, 23			
KI††					-19; 15;	34 a; 5*		
					-19, 15			
NaCl††	(001)	[100]	Mo	(111)	[110]	12, x	34 d; 6*	
				(331)		-21, x	34 d; 6*	
KCl††		[110]	Ag	(001)	[100]	30	9	
		[100]	Pd	(111)	[110]	-8, 13	-9	
			Ni	(001)	[100]	24	-9	
						15	-9	
MoS <sub>2</sub>	(0001)	[1010]	Ag**	(111)	[110]	9	35; 7**	
ZnS	(110)	[110]		(110)		25	35	
PbS	(100)			(100)	[001]	32	35	
FeS <sub>2</sub>						24	35	
Mica**	(001)	[100]	Au	(111)	[110]	10*	36; 4*, 7**	
		[010]				-4*	36; 4*	
		[100]	Ag			10*	36; 4*	
		[010]				-4*	36; 4*	
		[100]	Pd			7*	36; 4*	
		[010]				7*	36; 4*	
		[100]	Au			-5, x	36; 7**	
††CaCO <sub>3</sub> **			Ag			-5, x	36	
			Pd			-10, x	36	

††1. The potential troughs of the ionic crystals are assumed to be at the centres of the small squares (parallelograms for CaCO<sub>3</sub>) having at their corners alternately negative and positive ions.

2\*. This was shown to be the true orientation of deposit at the contact surface.

3\*. Three Ag (or Ni) atoms are fitted against two Na<sup>+</sup> ions.

4\*. Half of deposit units on potential crests.

5\*. The first misfit, e.g., -9, corresponds to orientation (a) the second, e.g., 29, to (b) and the third, e.g., -9, 29, to (c). See Fe on NaCl.

6\*. x = bad pattern and bad fit for a direction perpendicular to that for which the misfit is given.

7\*. There are indications that the orientation towards the contact surface is different from that in the bulk deposit.

TABLE III

CASES OF ORIENTED OVERGROWTH IN WHICH THERE APPEARS TO BE FIT IN ONE DIRECTION ONLY

*One-Dimensional "Fitting"*

Substrate			Deposit		Misfit %	Literature + Remarks
Au	(110)	[001]	Fe	(100) [001]	0, 30	5
Pd		[110]	PdO		[010] 11, 36	24
Ag	(001)	[100]	NaCl	(111) [110]	-3, 69	9
NaCl		[110]	{ Thio-urea	(010) [100]	8, 40	7 f
Mica		[010]		(100) [001]	-4, 50	7 f
		[100]	Urea		8, -50	7 f
NaNO <sub>3</sub>	(100)	[ds]**	NaCl	(111) [110]	-1, x	13; 1*, 2**
			KCl		10, x	13
			LiCl		-10, x	13
		[dl]**	KBr		-8, x	13; 2**
			KI		-1, x	13
NaNO <sub>3</sub>			NaI		10, x	13
(See also Tables I and II)						

1\*. x = bad fit, bad pattern.

2\*. ds = short face-diagonal, dl = long face-diagonal.

TABLE IV

RECORDED CASES OF ORIENTED OVERGROWTH FOR WHICH DETAILS ARE LACKING

Oriented overgrowth in the following cases has also been established :

*Royer*<sup>14c</sup>NH<sub>4</sub>Cl, NH<sub>4</sub>Br, LiNO<sub>3</sub>, KNO<sub>3</sub>, K<sub>2</sub>Zn(CN)<sub>4</sub>, K<sub>2</sub>Cd(CN)<sub>4</sub>, KPF<sub>6</sub>, RbPF<sub>6</sub>, CsPF<sub>6</sub>, NH<sub>4</sub>PF<sub>6</sub> on mica.*Willems*<sup>8k</sup>

A. Organic compounds on organic compounds.

Pentachlorophenol, aniline, pentabromphenol and anthracene on (001) of chloranil.<sup>8i</sup> $\beta$ -naphthol,  $\beta$ -naphthylamine, benzidine, anthracene, phenanthrene, fluorene, pyrene in molecular compound with hexachloronaphthoquinone on hexachloronaphthoquinone (unpublished).Coronene (uncertain whether in compound with picric acid) on (010) of picric acid.<sup>8g</sup>Anthracene on (010) of aminophenol.<sup>8j</sup>3-hydroxypyrene, *p*-nitrophenol, pentachlorophenol on (110) of urea, and pentachlorophenol on (010) of dioxopiperazine.<sup>8j</sup>

B. Organic compounds on metallic salts (and hydrates of metallic salts).

Succinic acid, *p*-aminobenzoic acid, pentachlorobenzoic acid on (100) of alkali halides, e.g., NaCl.<sup>8j</sup>Hexamethylenetetramine on (010) of gypsum.<sup>8g,d</sup>Pentachlorophenol, pentabromphenol on (100) of alkali halides.<sup>8i</sup> $\alpha$ -hydroquinone, *p*-hydroxydiphenyl, *p,p'*-dihydroxydiphenyl, 3-hydroxypyrene, pentachlorophenol on (100) of the carbonates of the calcspars series and NaNO<sub>3</sub>.<sup>8b,f,k</sup> $\alpha$ -hydroquinone and pentachlorophenol on a series of micas.Pentachlorophenol on (001) of Pennin and on KClO<sub>3</sub>, gypsum, anhydrite, bournonite.<sup>8i</sup>



condensing it on an independently heated substrate. With a suitable slit system a uniform, concentrated, direct molecular beam resulted. The arrangement of the source, slits and substrate is indicated by items 2 and 1 in Fig. 1.

The substrate (item 1) was mounted 25 mm. above the source in a copper frame which held it in place against an externally heated copper block. In this manner the lower face was exposed to the beam and maintained at the desired temperature by heating from the upper face. The temperature was regulated to 1 % by a proportionating potentiometer controller. The substrate consisted of a square plate of an ionic salt approximately 5 mm. on the side and 1 mm. thick. A freshly cleaved face was exposed just prior to a run. A thermocouple probe in contact with the lower face of the substrate indicated the film temperatures. Two additional platinum probes 2.0 mm. apart were in contact with the surface. The appearance of the first few layers of the film was indicated by the sudden decrease in resistance measured between the probes.

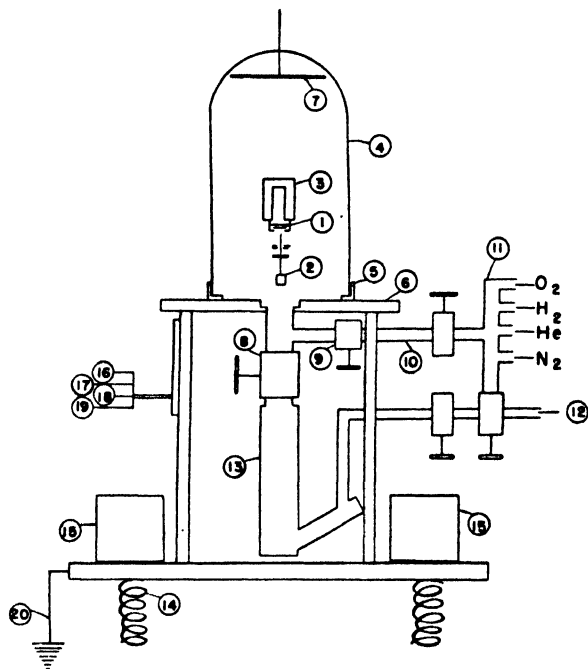


FIG. 1.—Vacuum evaporator: (1) Substrate; (2) Molecular beam source; (3) Furnace; (4) 18-in. glass bell-jar; (5) Right-angle neoprene gasket; (6) Polished steel plate; (7) High-voltage cathode-5000 V; (8) 4-in. packless valve; (9) 2-in. packless valve; (10) Manifold; (11) Purified gases; (12) Holding pumps; (13) High-capacity diffusion pump; (14) Shock mount; (15) 200-lb. weights; (16) Thermocouple pressure gauge; (17) Ionization gauge; (18) Temperature controller; (19) Electronic heater; (20) Electrical ground.

The source (item 2) of the beam was a small tantalum crucible located directly below the substrate. The microcrucible held a charge of 100 mg. The inside diameter of the crucible was 3 mm. but the beam was actually emitted through a 0.1 mm. orifice in a tantalum cap placed over the top of the microcrucible. The cap prevented splattering and also promoted thermal equilibrium of the atoms before they were emitted. The charge was outgassed by prefusing *in situ* before evaporating during which time the substrate was protected by an externally manipulated shield.

The heating of the crucible was very satisfactorily effected by an arrangement for direct electron heating indicated in Fig. 2. The crucible is shown with the lid off as item 1. Electrons emitted from an incandescent 30 mil. tungsten filament

(item 2) around the crucible are accelerated by a positive potential towards it. A tantalum shield (item 3) around the assembly reduced heat losses and another tantalum lid (item 4) shielded the substrate from direct exposure to the filament. The temperature of the microcrucible could be accurately adjusted and maintained at any temperature up to  $1500^{\circ}\text{C}$  within 2–3 % by regulating the filament emission and the accelerating potential. This high fidelity temperature control is necessary in the determination of the substrate temperature–condensation pressure relationship for various substrates. The substrate was tied in at the same potential as the crucible to eliminate the possibility that metal ions formed in or around the crucible may be spuriously accelerated towards the substrate.

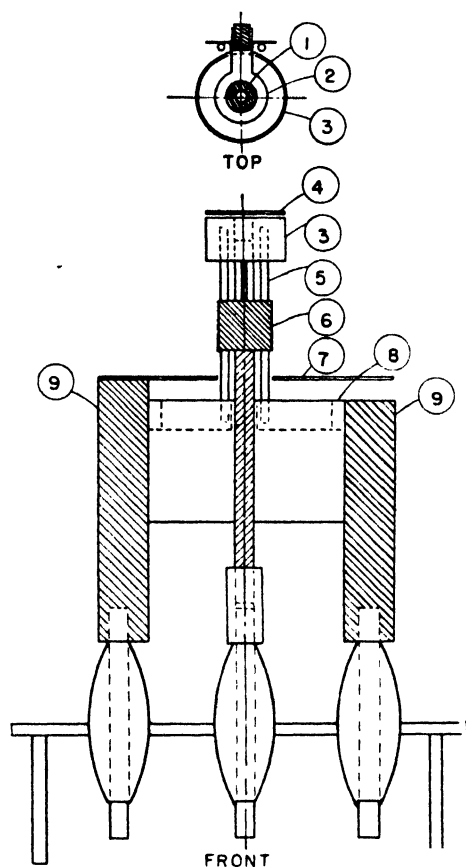


FIG. 2.—Electronic heater: (1) Tantalum crucible; (2) Tungsten filament; (3) Tantalum shield; (4) Tantalum lid; (5) Nickel leads; (6) Crucible electrode; (7) Stainless steel; (8) Mykroy spacer; (9) Filament electrodes.

In the measurement of critical condensation pressures the crucible temperature was slowly increased until the rate of evaporation of the aluminium was just enough to cause condensation on the substrate for a given base temperature. The rapid decrease in the film resistance between the probes was used to indicate the formation of the initial layer. The process was reversible, that is, a small increase in base temperature for a critical pressure caused the film to evaporate. The microcrucible could be readily removed from the crucible electrode (item 6) for replacement and the position could be easily lined up by adjusting the eccentric at the base. The crucible temperature was measured by a Chromel-Alumel thermocouple mounted in the bottom.

**RATE OF CONDENSATION.**—The condensation rate for a given crucible and substrate temperature was determined by weighing *in situ* a thin glass slide hanging on a quartz fibre over the molecular beam as indicated in Fig. 3. A sensitive quartz torsion microbalance facilitated accurate and quick measurements in vacuum. The torsion fibre was rotated by magnetic coupling through the glass wall. The sensitivity of the weighing was  $10^{-6}$  g. with a 23 m $\mu$  quartz torsion fibre (item 5). The thickness corresponding to weight increments was calculated for a constant area assuming the film to be flat and continuous and the film density to be comparable to the mass density. A microgram corresponding to a hundred ångströms thick layer of aluminium for the film was used. The condensation rates for various substrate and crucible temperatures were calibrated in this manner.

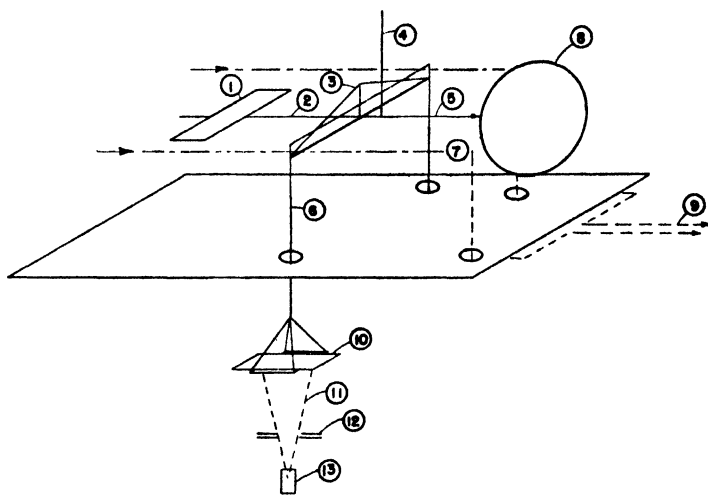


FIG. 3.—Quartz torsion microbalance: (1) Bow fibre; (2) Static; (3) Beam; (4) Hang-up; (5) Torsion fibre; (6) Hang-down; (7) Image of index; (8) Graduated wheel; (9) Single field image; (10) Substrate; (11) Molecular beam; (12) Collimating slit; (13) Source.

**EXAMINATION OF THE FILM: Metallography.**—Upon completion of the run the substrate was cooled *in situ* and removed for examination. Reflectivity of the surface varied from very mirror-like to cloudy as the film thickness and substrate temperature increased. Metallographic examination of the samples was considerably hampered by their fragility. Where it was possible to observe grains without destroying the film the average grain size was two to five thousand ångströms for a film of the same thickness.

**Structure Determination.**—The film structure was determined with X-ray diffraction using a surface reflection pinhole technique in a vacuum camera as indicated in Fig. 4. A Picker-Waite diffraction unit was used with a water-cooled chromium target. The exposure time varied from 2 to 15 hr. for film thicknesses from 5000 to 500 Å with an accelerating potential of 50 kV and a space current of 10 mA. The sample (item 6) was anchored flat on the turntable (item 7) and rotated around an axis (item 19) normal to the surface of the sample. The axis of rotation was inclined away from the incident beam an amount corresponding to the Bragg angle for reflection from the plane of preferred orientation. The camera was particularly designed to suit the geometry and orientation unique to the samples studied. The simplicity of the film patterns indicated in Fig. 5 *a* and Fig. 5 *b* clearly show the advantage obtained. Preferred orientation is characterized by segmentation of the lines into local marks as shown by the heavy marks of five degrees length on the dashed reflection lines in Fig. 5 *b*. The vertical distance from the centre line measures the orientation azimuths

characteristic of a preferred orientation. The value of the orientation azimuths ( $\varphi$ ) can be calculated for any preferred orientation.<sup>2</sup>

$$\cos \rho = \cos \beta \sin \theta + \sin \beta \cos \theta \cos \varphi \quad (1)$$

where

- $\rho$  = angle between oriented plane and reflecting plane,  
 $\beta$  = angle between normal to oriented plane and incident beam,  
 $\theta$  = Bragg angle for reflection from reflecting plane,  
 $\varphi$  = orientation azimuth for oriented plane.

Some values of  $\varphi$  calculated for reflection of  $K_{\alpha}$  and  $K_{\beta}$  chromium radiation from the (111), (200) and (311) planes of aluminium for (111), (100) or (110)

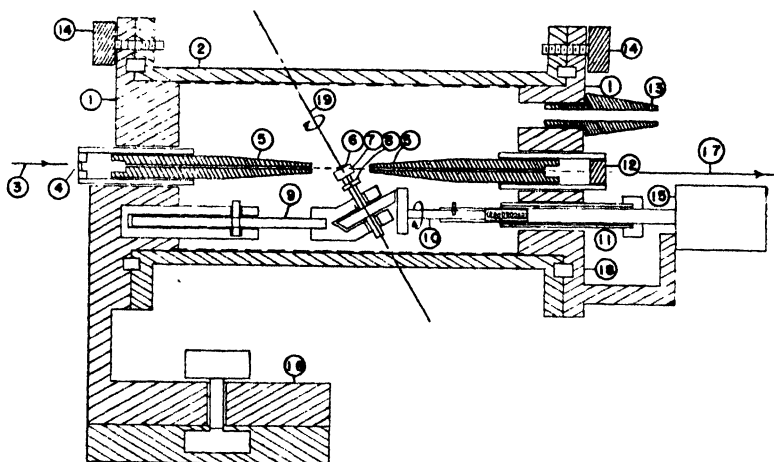


FIG. 4.—X-ray vacuum camera: (1) End plates; (2) Film case; (3) Entrance beam; (4) Beryllium window; (5) Pinholes; (6) Specimen; (7) Lucite spacer; (8) Lock-nut; (9) Rotor assembly; (10) Drive-wheel shaft; (11) Rotor vacuum seal; (12) Lead-glass window; (13) Vacuum outlet; (14) Plate nuts; (15) Motor; (16) Track clamp; (17) Exit beam; (18) Neoprene gaskets; (19) Axis of specimen rotation.

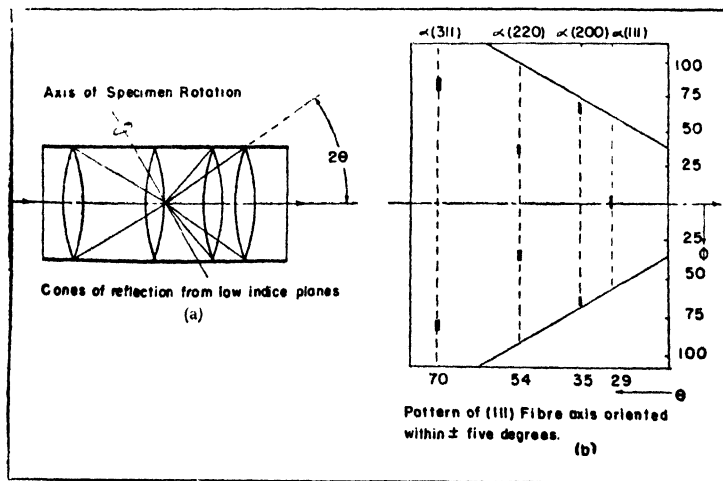


FIG. 5.—Diagram of X-ray pattern from oriented film.

<sup>2</sup> Barrett, *Structure of Metals—Crystallographic Methods, Principles and Data* (McGraw-Hill, New York, 1943), p. 156.

orientation are listed in Table I. The kind of orientation can be readily determined from the pattern defined by the characteristic values of  $\varphi$ . A semi-quantitative value for the degree of orientation with a maximum error of 10 % in this determination can be obtained by measuring the opaqueness of the spot relative to the integrated opaqueness of the whole line with a Leeds and Northrup recording microphotometer. The error is introduced by the assumption that the intensity at any one spot on the film is linearly proportional to the amount of radiation reflected to that point and is the same for all azimuth angles.

TABLE I  
BRAGG ANGLES AND ORIENTATION AZIMUTHS  
CHROMIUM RADIATION ON ALUMINIUM

Radiation CrK	Reflecting Plane	Bragg Angle ( $\theta$ )	(111)	Orientation Azimuths ( $\varphi$ ) (110)	(100)
$\alpha$	(111)	29.5	0, 83	41,109	64
$\beta$	(111)	26.8	0, 80	39,105	62
$\alpha$	(200)	34.7	68	55,119	0, 119
$\beta$	(200)	31.0	65	53,111	0, 111
$\alpha$	(220)	54.4	63	0, 118	82
$\beta$	(220)	47.0	52	0, 94	68
$\alpha$	(311)	70.0	95	104	89
$\beta$	(311)	59.2	58,142	63	50

## Results

**Orientation Results :** GENERAL.—A quantitative dependence of degree of preferred orientation on film thickness and substrate temperatures was found over a considerable range of thickness and temperature for eleven aluminium-substrate pairs. It was necessary, however, to make a preliminary evaluation of four other factors sufficiently well so as to minimize their influence. The pertinent results of the preliminary survey is herewith presented in condensed form as a background against which the significance of the quantitative aspects can be more intelligently considered.

**Film Growth Rate.**—Foremost is the important influence of film growth rate on structure. Since the experimental system was not propitiously suited for studying this aspect it was maintained at a constant value in all experiments. The evaporation rate was adjusted for each substrate temperature corresponding to an effective film growth rate of ten to thirty monolayers of aluminium per second. In the case of the binding energy determinations, however, the film growth rate was not controlled since it was only desired to determine the condition for minimum condensation.

**Heat Treatment.**—Heat treating of the substrates with or without adherent metal film caused no striking change in the resulting orientation. The films were therefore usually kept at constant temperature during formation and then permitted to cool by radiation in a vacuum. Annealing a randomly oriented film at elevated temperatures (up to 600° C) in helium resulted only in grain growth. Likewise oriented structures were not markedly altered by annealing under similar conditions. This temperature stability of the structure is in contrast to the temperature sensitive orientations of thinner aluminium films (400 Å) previously reported.<sup>3</sup>

**Gas Atmosphere Effects.**—The influence of gases present even at  $10^{-6}$  mm. pressure was also considered. The pressure of purified quantities of helium, oxygen, nitrogen and hydrogen at low pressures ( $10^{-4}$  mm.) decreased the orientation to a relatively small extent. The effect was not unique to any one gas or substrate and appeared solely to hamper the steady evolution of aluminium

<sup>3</sup> Dixit, *Phil. Mag.*, 1933, **16**, 1049.

vapour. In no case was a gas such as helium observed to improve the film orientation as reported by others for thinner films.<sup>4</sup>

*Substrate Condition.*—The contamination of the substrate surface itself by the gases previously listed was not considered to be critical. Oriented films could be formed on freshly cleaved rocksalt which had been preheated in oxygen and hydrogen atmospheres. In all cases, however, the cleanliness of the substrate was a critical requirement and best results were always obtained with freshly cleaved ionic surfaces whose lattices had an arrangement of ions, the geometry and dimensions of which showed a certain correlation to that of aluminium. The limited number of such salts emphasized the preparation of oriented substrates by other means. Even after repeated polishing, etching and annealing, use of NaCl and LiF surfaces obtained in this manner was only moderately successful. Successful techniques for exposing any desired substrate orientation in a suitably clean and flat condition would be most useful for further studies.

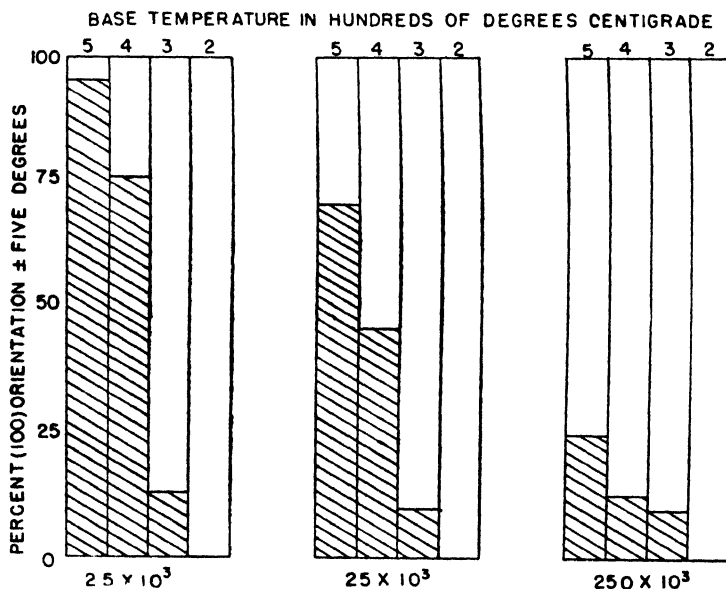


FIG. 6.—Approximate film thicknesses in Angströms. Orientation of aluminium film deposited on cleavage face of sodium chloride. Each column indicates average values for four samples. Dev.  $\pm 10\%$ .

**SPECIFIC FACTORS: Film Thickness.**—Initial studies made it evident that degree of orientation was very dependent on film thickness for all film-substrate pairs. This characteristic will be described first since it depended only on temperature and film thickness and was general to all substrates. The %-orientation for films on the (100) face of rocksalt for various thicknesses and base temperatures is plotted in Fig. 6 as an illustration. An exponential dependence of orientation on film thickness was observed and the data plotted in Fig. 7 is a typical case. It is interesting to note that a critical film thickness for perfect orientation at each temperature is suggested by extrapolation of the straight line to small film thicknesses. A striking temperature dependence is also indicated by the distinctly small slope of Curve 2 compared to Curve 3 in Fig. 7. The validity of a strong base temperature dependence of %-orientation is also indicated in Fig. 8 for a variety of film-substrate pairs. Discussion of the temperature dependence is, however, temporarily postponed until right after the discussion of the thickness effect.

<sup>4</sup> Beeck, Smith and Wheeler, *Proc. Roy. Soc. A*, 1940, **62**, 177.

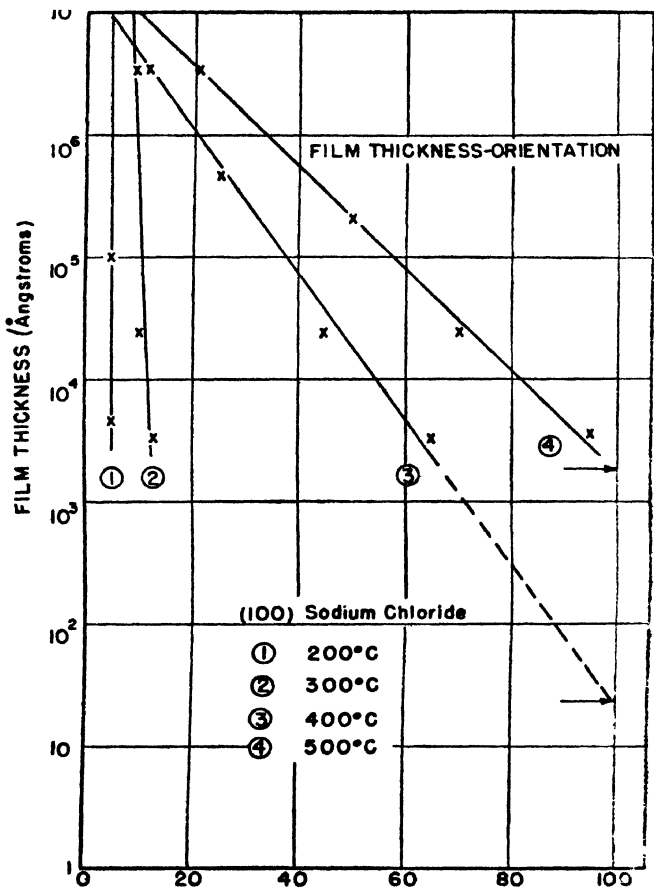


FIG. 7.—Per cent. orientation.

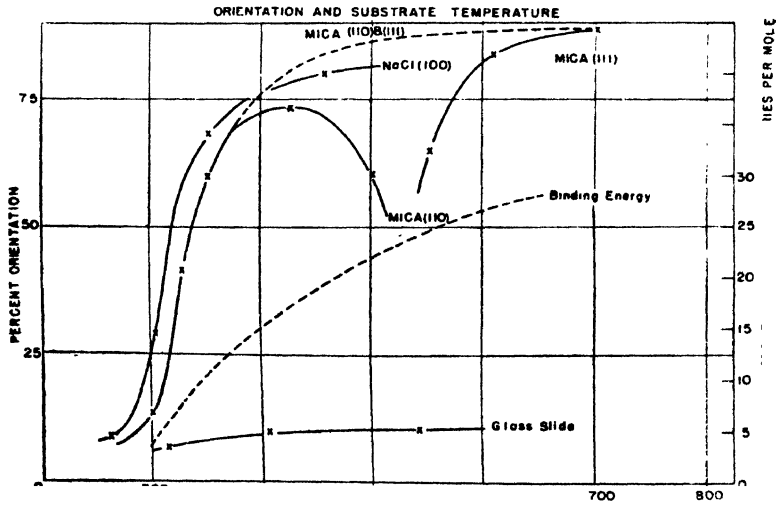


FIG. 8.—Substrate temperature—centigrade.

The thickness dependence may mean that the oriented arrangement is constant at all points in the film and merely decreases as the film thickens. It seems more reasonable to assume that the orientation is strongly dependent on the substrate and produces an orientation large at the inner film surface and decreasing towards the outer film surface. The latter possibility is in agreement with the surface reflection characteristics of X-rays. In this case a disproportionately large fraction of the radiation producing the pinhole pattern is reflected from the outer planes of the film. A quantitative illustration of this can be presented by calculating the intensity of radiation reflected from successive layers of metal atoms as follows.<sup>5</sup> The intensity ( $I_t$ ) reflected from a thickness ( $t$ ) relative to the intensity ( $I_d$ ) reflected from an infinitely thick film can be expressed :

$$\frac{I_t}{I_d} = \frac{\int_0^t I_2 dt}{\int_0^\infty I_2 dt} \quad (2)$$

where

$$I_2 = k I_0 \exp - 4 \left( \frac{\mu}{\rho} \right) \rho \left( \frac{td}{\lambda} \right) \quad (3)$$

neglecting the scattering of the ray after it has emerged from the sample. Here  $I_2$  is the final intensity,  $I_t$  the intensity upon reflection from a diffraction volume element at a depth  $t$  below the surface, and  $I_0$  is the incident intensity before entering the sample. The term  $(4td/\lambda)$  is a linear expression of the total distance travelled on the sample when the ray penetrates a depth  $t$  and undergoes a Bragg angle reflection. The other symbols are :

- $k$  = efficiency constant, unity,
- $I_0$  = initial intensity of incident beam,
- $(\mu/\rho)$  = mass absorption coefficient,
- $\rho$  = film density,
- $d$  = interplanar distance of oriented planes,
- $t$  = film thickness penetrated,
- $\lambda$  = wavelength of X-ray radiation.

Values of ( $I_t/I_d$ ) for a limiting thickness of  $5 \times 10^5 \text{ \AA}$  have been calculated from eqn. (2) for successive depths of penetration into the sample. The amount of radiation from each layer characterizes the degree of orientation in that layer and the over-all variation of apparent orientation with film thickness indicates the decrease of orientation with increasing film thickness. The calculations were made for the  $K_\alpha$  radiation from a chromium target diffracted by the (100) oriented planes of aluminium. The results in Table II are presented: column 1, depth of penetration ( $\text{\AA}$ ); column 2, thickness penetrated relative to limiting thickness; and column 3, the corresponding %-intensity for that thickness penetrated over the total radiation recorded on the film.

Although the pinhole pattern is an integrated effect of orientation through the entire layer, it is obvious that the pattern is a weighted average heavily in favour of the extreme surface. For an example, with a chromium target twice as much radiation is reflected from the outer half thickness than the inner half thickness of an aluminium film 5000  $\text{\AA}$  thick. Hence in the film thickness study

TABLE II  
DISPROPORTIONATE VARIATION OF  
INTENSITY OF DIFFRACTED X-RAY  
RADIATION WITH DEPTH OF PENETRATION

(1)	(2)	(3)
$t(\text{\AA})$	$\frac{t}{t_d}$	$\frac{\% I_t}{I_d}$
$5 \times 10^2$	1	9
$5 \times 10^3$	6	24
$5 \times 10^4$	25	53
$1 \times 10^5$	50	76
$5 \times 10^5$	100	100

<sup>5</sup> Hess (Institute for the Study of Metals) (private communication to the author).



the orientation of the outer surface was essentially observed. The orientation is greatest in the region nearest to the substrate-film interface.

*Substrate Temperature.*—A base temperature dependence in which orientation of the film increased rapidly at some characteristic temperature for each substrate illustrated in Fig. 8 was typical of all substrates. The less the maximum orientation, however, the smaller the dependence on the characteristic temperature. This is illustrated by the contrast in the curves for (100) orientation on rocksalt and glass. The temperature dependence may mean that the metal atoms must possess a minimum kinetic energy corresponding to the observed temperature for maximum orientation for them to take up the preferred positions suggested by the substrate. The transition of a (110) orientation of the film on mica at low temperatures to a (111) orientation at higher temperatures indicates that a higher minimum mobility is required for formation of the second configuration. In all cases the rate at which orientation increased with base temperature as well as the maximum value it approached was typical of the substrate. It indicates that production of ordered arrangements is governed not only by the interaction of the substrate and metal but by a relatively slow temperature-dependent surface diffusion process as well. Calculation of activation energies for the rate process involved seems premature until the mechanism of arrangement is better defined. The characteristic values of base temperature and maximum observed orientation are plotted in Fig. 8 for rocksalt, mica and glass, and listed for eleven substrates in columns 2 and 4 of Table III.

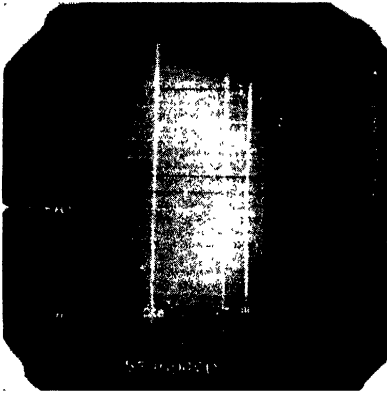
TABLE III  
STRUCTURE CHARACTERISTICS OF THIN ALUMINIUM FILMS

Substrate	Temp. Orient. °C	Direct. Orient.	% Orient.	Pressure $\times 10^4$ cm.Hg.	$A$ kc./m.	$\Delta$ kc./m.	$A - \Delta$ kc./m.	$E_c$ kc./m.
Mica ..	600	(111)	87	0.007	42	20	22	22
Mica ..	450	(110)	75	1.52	28	10	18	20
NaCl ..	350	(100)	80	0.003	31	15	16	18
NaCl ..	350	(110)	50	0.024	21	10	11	12
LiF ..	400	(111)	55	0.022	33	20	13	10
LiF ..	300	(100)	50	0.012	25	15	10	10
CaCO <sub>3</sub> ..	300	(111)	15	0.012	25	20	5	5
Glass ..	400	(100)	10	6000	15	15	0	—
CaF <sub>2</sub> ..	300	(111)	10	760	20	20	0	2
ZnS ..	300	(111)	10	700	21	20	1	4
Sodalite..	300	(111)	10	700	21	20	1	2

Experimental data for mica in the temperature region intermediate between the (110) and (111) orientations were inconclusive. It is noteworthy that the most oriented configurations corresponded to the higher orientation temperatures. This characteristic is a general one for all the substrates studied. It is illustrated by the dashed curve in Fig. 8 in which the orientation temperature as abscissa is plotted against the substrate binding energy as ordinate on the right. The substrate binding energy, heretofore undefined, is described in a subsequent section and shown to be proportional to the percentage of the observed film orientation

*Nature of Substrate.*—From the facts presented so far it seems clear that the nature and degree of the observed orientation was critically dependent on the substrate. This dependence held in general for all the substrates studied. In every case there was a correlation of some kind between the geometry and dimensions of the underlying lattice and that plane of aluminium preferentially oriented parallel to it. For example, the (100) face of aluminium was the only orientation observed on the (100) face of the alkali halide substrates. Similarly the (111) planes of aluminium tended to be preferentially oriented parallel to substrates with hexagonal cleavage or hexagonal-polished faces, providing the base temperature and film thickness were favourable. In other cases (110)





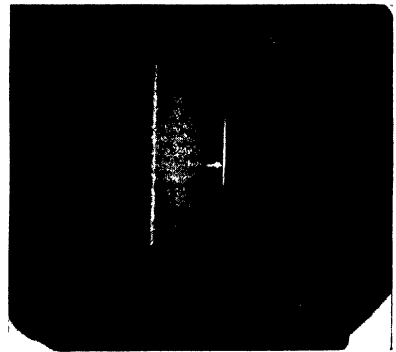
(a) Aluminium on glass random orientation.  $10^4$  Å thick.



(b) Aluminium on (100) sodium chloride 80 % (100) orientation  $1.2 \times 10^3$  Å thick.



(c) Aluminium on (111) mica 87 % (111) orientation.  $1.3 \times 10^3$  Å thick.



(d) Aluminium on glass 10 % (100) orientation.  $10^3$  Å thick.

FIG. 9.

orientation was observed to occur on (110) oriented substrates. This is not a general effect, however, since cases occur where substrates stabilize preferred film orientations other than their own but in most cases that orientation of aluminium occurred for which the geometry and spacing of the metal atoms yielded the best fit on the substrate. The data are summarized in columns 1, 2, 3, 4 of Table III in order of decreasing orientation. The direction of orientation in the film listed in the third column is the same as that of the substrate for the first seven items. A small orientation was observed on glass which, of course, possesses no definable surface arrangement. The small degree of orientation on fluorite was barely measurable. The (111) orientation on zinc blende and sodalite was also very small. The last two substrates possess cubic lattices with good (110) cleavage faces and are examples of film orientation differing from that of the substrate.

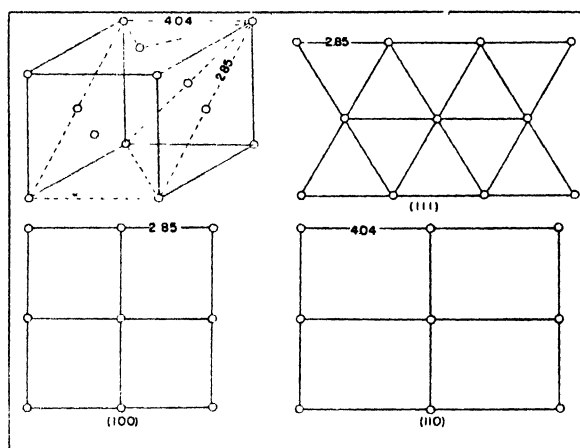


FIG. 10.—Aluminium unit cell.

Some typical surface reflection X-ray film patterns are illustrated in Fig. 9. The interpretation is handicapped by the poor contrast of the aluminium lines, the strong and complex pattern caused by reflection from the substrate and the poor reproduction, but the over-all pattern analysis is quantitatively unique for each orientation. Continuous reflection from all the low indice reflection planes indicate complete randomness in Fig. 9 *a*. The sharply defined orientation of a (100) aluminium film on rocksalt is illustrated in Fig. 9 *b*. Fig. 9 *c* is greatly complicated by the mica pattern but arrows indicate the discrete aluminium reflections corresponding to (111) orientation. A poorly oriented film on glass tending towards (100) orientation is included for comparison (Fig. 9 *d*).

### Discussion

**Geometric Considerations.**—The good correlation between substrate and film orientation is in accord with the excellent matching between the oriented aluminium plane and the geometry and dimensions of the surface lattice upon which it forms. Similar results have been reported for thinner films.<sup>6</sup> The striking geometric kinship among the three low indice planar arrangements, (100), (110), (111), of aluminium in Fig. 10 and the geometry of the corresponding planes in sodium chloride and lithium fluoride in Fig. 11 suggests that such correspondence between film and substrate promotes a related orientation in the former. This hypothesis is suggested by the

<sup>6</sup> Bruck, *Ann. Physik*, 1936, **26**, 233. Rudiger, *Ann. Physik*, 1937, **30**, 505. Finch, Quarrell and Wilman, *Trans. Faraday Soc.*, 1935, **31**, 1051.

Table of lattice distances summarized in Fig. 11. They agree within a few % in each case except for the (111) sodium chloride face, for which the lattice spacing is 40 % greater than the corresponding spacing in the (111) aluminium plane. It is doubtful whether this correlation is generally essential for substrate-metal interaction but it is significant that no (111) orientation of aluminium was ever observed on a (111) sodium chloride surface. Corresponding orientations were observed in every other case.

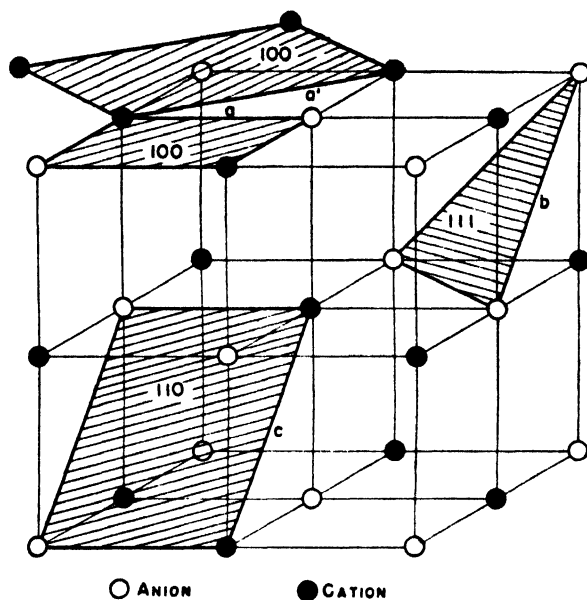


FIG. 11.—Unit cell of alkali halide.

		Lattice parameters (Å)		
		Al	NaCl	LiF
(100) <i>a</i>	..	2.85	.. 2.83	.. 2.84
(111) <i>b</i>	..	2.85	.. 3.98	.. 2.84
(110) <i>c</i>	..	4.04	.. 3.98	.. 4.01

Substrates with hexagonal cleavage faces of atomic dimensions corresponding to the (111) face of aluminium yielded (111) oriented aluminium films. The fairly complicated surface structure of mica accommodated (110) arrangement of aluminium as well. The arrangement of the atoms in the hexagonal cleavage faces of mica, calcite and fluorite are drawn to scale in Fig. 12. The matching of lattice distances was poorer than for the cubic face cleavage substrates and the observed degree of orientation was also correspondingly poorer with the exception of mica. The (110) cleavage faces of cubic zinc blende and sodalite are not indicated, but the matching was relatively poor for both substrates and they are unsatisfactory as (110) directing surfaces.

In all cases studied, the nature and degree of the observed film orientation bore a close relationship to the geometry and dimensions of the underlying substrate. It appears that directing forces are geometrically distributed on the substrate surfaces in close correspondence to the atomic distribution in the substrate plane. An interpretation based on this approach will be discussed in the next section.

**Characterization of Substrate.**—In an effort to characterize the substrates an effect, discovered by Wood<sup>7</sup> and studied by Estermann,<sup>8</sup> was used in a modified form. When a beam of metal vapour is directed at a heated substrate, condensation will occur if the pressure is sufficiently high or the substrate temperature sufficiently low. Whether most of the atoms bounce off the surface losing none or relatively little of their kinetic energy or whether they are accommodated on the surface depends on the relative values of the aforementioned variables plus a third, the attraction of the substrate for the metal atoms. Since the relationship between these factors can be quantitatively expressed, the attraction of the substrate may be determined providing the corresponding pressures and base temperatures can be measured. This pressure-temperature dependence was determined for all the substrate-metal pairs at those substrate temperatures at which maximum orientation was known to occur in each case. The substrate temperature was measured with a thermocouple probe on the surface. The metal pressure was not measured directly but calculated from the crucible

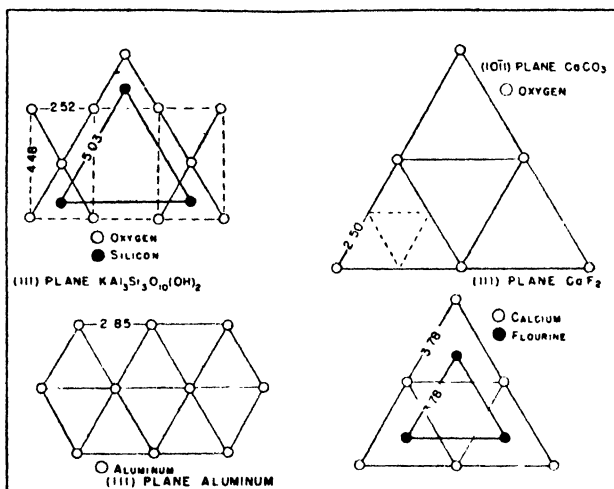


FIG. 12.—Crystal substrates with hexagonal cleavage.

temperature using the free energy of vaporization values for aluminium.<sup>9</sup> The minimum vapour pressure was accurately determined for each base temperature at which condensation of the first layers took place. The formation of the first layer was indicated by measuring the sudden drop in film resistance between two probes on the surface. The corresponding values of pressure and base temperature for a group of typical runs are plotted in Fig. 13. The pressure-temperature relationship can be expressed as

$$p = a_1 e^{-A/RT} \quad (4)$$

where

$p$  = pressure of metal vapour,  
 $a_1$  = constant, insensitive to temperature,  
 $T$  = absolute temperature of the substrate,  
 $A$  = an energy term, characteristic of the film and the substrate.\*

<sup>7</sup> Wood, *Phil. Mag.*, 1916, **32**, (6), 365.

<sup>8</sup> Estermann, *Z. Elektrochem.*, 1926, **31**, 441.

<sup>9</sup> Kelley, *The Free Energies of Vaporization and Vapour Pressures of Inorganic Substances* (Bulletin 383, Bureau of Mines, 1935).

The values of  $a_1$  could be interpolated from the intercept on the pressure axis of the curves plotted in Fig. 13. It is insensitive to the nature of the substrate and to the base temperature for the conditions observed. Hence, it was of little use for characterizing the substrates on a relative basis. It, however, includes at least three significant terms describing: (a) geometry of the system, (b) size of the condensing particles, (c) a linear temperature correction. Hence, an interpretation of the mechanism of condensation would eventually require an analysis of  $a_1$  into its component terms.

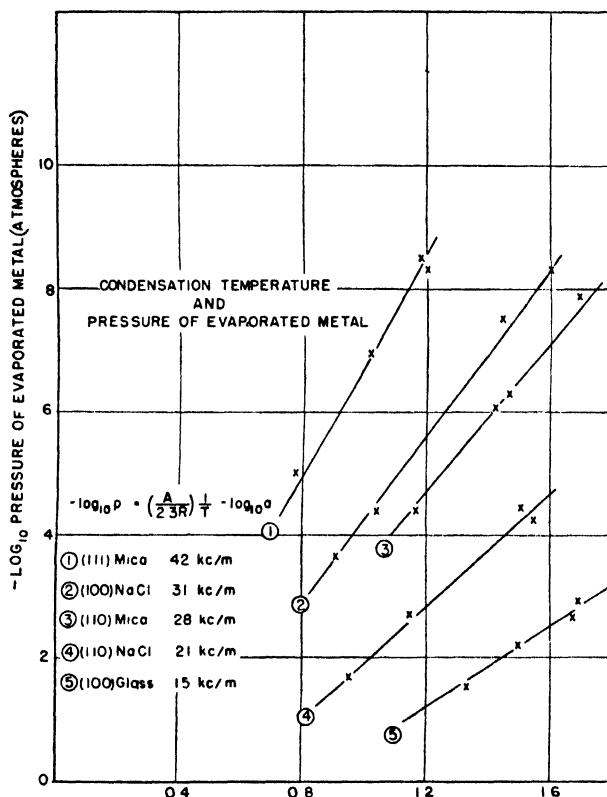


FIG. 13.

$1/T \times 10^3$  degrees (absolute).

The values of  $A$  could be readily interpolated from the slopes of the straight lines plotted in Fig. 13. Some typical values are listed there in order of decreasing magnitude. Corresponding values of temperature, pressure and  $A$  are more completely listed in columns 2, 5 and 6 of Table III.

A definite trend of  $A$  in Table III from 42 kc./m. to 20 kc./m. is evident. This trend corresponds to decreasing observed orientation. It is significant that the values of  $A$  are smallest for the ionic substrates at the end of the Table for which the orientation was poorer. The smaller surface-free energy of the (111) arrangement corresponds to the observation that the  $A$  values for (111) substrate-film orientations are somewhat greater than those for (110) and (100) orientations for each substrate-metal pair.

An interpretation of  $A$  as describing the heat of condensation of the

first layer of metal atoms on the substrate is indicated by the temperature-dependence relationship established by the straight lines in Fig. 13 and by the empirical findings of Wood and Estermann. A theoretical analysis of their work by Semenoff<sup>10</sup> applied to these results indicates that

$$A = E + \Delta, \quad (5)$$

where  $E$  = the adhesive energy of binding of the metal and substrate,  
 $\Delta$  = the energy of binding of the aluminium atoms in the first layer, e.g., the surface energy, characteristic of the metal film itself.

If the first term is large the substrate is likely to influence strongly the formation and arrangement of the atoms in the first layer providing the atoms possess sufficient mobility to assume those positions on the surface of lowest potential energy. If it is small, relative to the second term, that is, the adhesive forces between metal and substrate are negligible compared to the cohesive binding between metal atoms, the film formation will be relatively independent of the substrate and, should any orientation occur, it will be that arrangement for which the surface-free energy is smallest. Formation of an oriented first layer under the first condition would facilitate the occurrence of the same orientation for subsequent layers. The degree of observed orientation should increase with the value of  $E$  providing other factors are also favourable, e.g., mobile atoms and relatively thin films. Formation of an oriented layer under the second condition may also occur but the degree of orientation will likely be considerably less. It is noteworthy that the values of  $A$  varied from 42 kc./m. for aluminium on mica to 15 kc./m. for aluminium on glass (column 6, Table III). In the latter case one might consider the interaction between the glass and the metal to exert a relatively small influence on the film structure and the measured heat of condensation to correspond mainly to the cohesive forces in the (100) plane of aluminium. Since there are about one-third the number of bonds in this configuration compared to that of massive aluminium, the surface energy can be roughly approximated to be one-third of the molar heat of vaporization or 22 kc./m. For this crude approximation the order of magnitude agrees with the experimentally determined value measured on an amorphous substance like glass. Neglecting the entropy correction, the substrate binding energy for the other substrate-metal pairs may be similarly approximated by subtracting an energy,  $\Delta$ , corresponding to the cohesive binding energy of the film, from  $A$ , the total energy of condensation. In view of the assumptions involved the values obtained are speculative but the resulting values ( $E$ ) listed in column 8 of Table III are of the right order of magnitude. These approximations compare favourably with values calculated on the same basis as van der Waals' interaction. The trend of the experimental values of  $E$  is in qualitative accord with the trend of observed orientations for each metal-substrate pair. This is indicated by the data on the maximum orientation and the substrate binding energies listed in columns 4 and 8 in Table III. The correlation is also evident in Fig. 14 in which the maximum observed orientation is plotted as ordinate against the substrate binding energy as abscissa. The calculated values, included for comparison, are now discussed.

**Van der Waals' Interaction.**—Understanding of the binding between a metal and an ionic surface would provide considerable insight as to the nature of the metal-substrate interaction. A rigorous attempt to define the binding is well beyond the scope of this paper, but some speculation in this direction seems justified. The characteristic of the binding, namely,

<sup>10</sup> Semenoff, *Z. physik. Chem. B*, 1930, **7**, 471.



its relative magnitude and non-specificness, suggests the validity of an approach based on van der Waals' interaction between the first layer of metal and the substrate. An analysis similar to a certain extent to the calculation of heats of adsorption of gases physically adsorbed on ionic surfaces near the boiling point of the gas seems justified. It is obvious that the chief distinguishing characteristic between metal and physically adsorbed gas films, other than the different temperature range in which they form, is the marked importance of the cohesive forces in the former case. It is conceivable, nevertheless, that a strong periodicity in the potential energy surface of the substrate towards the metal atom may be sufficient to start the condensation in a favoured direction. The energy of van der Waals' binding of aluminium on each of the substrates was calculated on this basis.

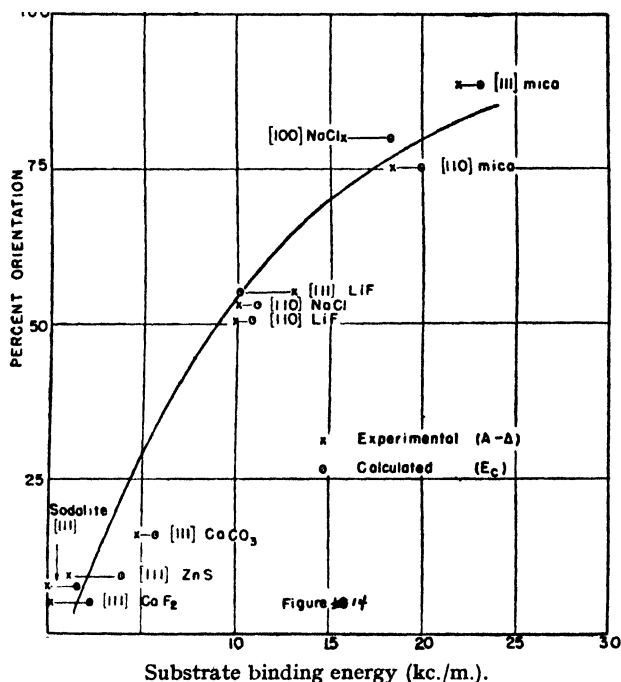


FIG. 14.—Substrate orientation.

Van der Waals' interaction between non-polar molecules has three important constituent parts: (1) the attraction between fluctuating dipole and induced dipole (dispersion effect), varying inversely as the sixth power of the distance, (2) the attraction between fluctuating quadrupole and induced dipole varying inversely as the eighth power of the distance and (3) the repulsion energy decreasing exponentially with the distance. A fourth constituent part is unique to ionic surfaces: the so-called influence effect. The latter is due to the fact that the charged ions of the substrate induce a dipole moment in the metal atom, which results in an attraction between the ions and the induced dipole. At the equilibrium distances characteristic of the metal films, the first of the four terms is by far the most important. The calculations were made on an approach similar to Orr<sup>11</sup> in which he calculated heats of physical adsorption of argon on potassium chloride.

<sup>11</sup> Orr, *Trans. Faraday Soc.*, 1939, **35**, 1247.

The dispersion effect was introduced by London<sup>12</sup> in the calculation of heats of adsorption. The dispersion potential  $\varphi$  between an atom of metal and an ion of the substrate can be written

$$\varphi = -C/r^6, \quad (6)$$

where  $r$  is the equilibrium distance, and  $C$ , the dispersion constant, is given by

$$C = \frac{3}{2} \alpha \alpha' \frac{JJ'}{J + J'}, \quad (7)$$

where

$$\begin{aligned} \alpha &= \text{polarizability of the metal,} \\ \alpha' &= \text{polarizability of the ion,} \\ J &= \text{characteristic energy of the metal,} \\ J' &= \text{characteristic energy of the ion.} \end{aligned}$$

The interaction between an atom and the entire surface of the substrate can be very simply calculated if one assumes that the distance between atom and ion is not smaller than the distance between ions. In this case the summation over the ions of the substrate can be replaced by an integration. For alkali halide substrates this approximation will yield values that are too low by 25 % to 30. %. For the mutual dispersion energy of an infinitely large surface and an isolated atom,

$$\varphi = - \int \frac{C}{r^6} N dv = - \frac{N\pi C}{6r^3}, \quad (8)$$

where  $N$  = number of ions per cm.<sup>3</sup> and  $dv$  is the volume element. Substituting expression (6) for the dispersion constant,

$$\varphi = - \frac{N\pi}{4} \frac{\alpha \alpha'}{r^3} \frac{JJ'}{J + J'}. \quad (9)$$

An exact evaluation from eqn. (8) is not possible because some of the experimental data are missing, particularly the value of  $J$  for aluminium. Nevertheless, to show the order of magnitude, calculations were made using the first ionization potential. The value of  $N$  for the substrates other than the alkali halides was calculated from the density. The distance  $r$  between an ion and an aluminium atom was assumed to be made up of two parts after London,<sup>13</sup> viz.,

$$r = d_1/2 + d_2/2 \quad (10)$$

For  $d_1/2$  half the distance between ions in the substrate was used and for  $d_2/2$  half the interplanar distance for that plane of aluminium observed to be preferentially oriented. The identity and geometry of the important ions in the substrate were not always definitely established and a choice had to be made in some cases. The ion was chosen whose arrangement on the surface best fitted the observed aluminium orientation. For example, the oxygen ions were chosen, instead of the silicon ions, in mica. The calculated binding energy for both is listed in Table IV for comparison. The atomic polarizabilities were taken from Van Vleck<sup>14</sup> if possible, or calculated from

$$\alpha = \frac{e^2}{4\pi^2 m \nu^2}, \quad (11)$$

where  $\nu$  is the characteristic frequency of the atom and the other symbols have the customary significance. The polarizability and characteristic energies

<sup>12</sup> London, *Z. physik. Chem. B*, 1930, **11**, 222.

<sup>13</sup> London, *Z. physik. Chem. B*, 1930, **11**, 222.

<sup>14</sup> Van Vleck, *Electric and Magnetic Susceptibilities* (Oxford University Press, 1932), p. 225.

for the ions of the alkali halides were taken from Mayer's<sup>15</sup> analytical treatment of the lattice energy characteristics of alkali halides. The validity of the physical constants in this case warranted more extended consideration. A potential energy surface for the system (100) aluminium-sodium chloride was constructed after Orr.<sup>16</sup> It is schematically represented in Fig. 15. The potential hole in the centre represents a position of the aluminium in which the potential energy is 7 kc./m. lower than a position over the cation. The position over the anion corresponds to the highest potential energy on the surface. For this system it is evident that the central site is relatively large but is deeper by 7 kc./m. than the next most favourable site and corresponds to a binding energy of approximately 18 kc./m. It is noteworthy that the atoms of the (100) aluminium plane could be laid over the grid

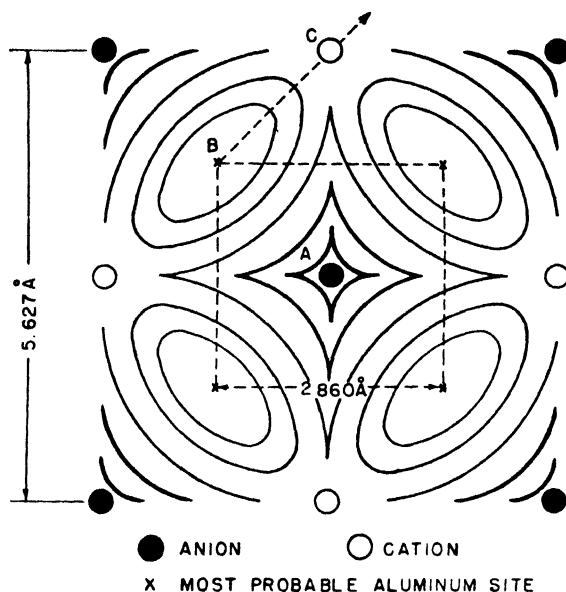


FIG. 15.—Potential energy surface (100) face sodium chloride unit cell.

formed by the potential energy holes with negligible distortion. The calculations are summarized in Table IV, in which the atom positions, the ionic polarizabilities, the characteristic energies, the dispersion constants and the calculated substrate binding energies are tabulated for thirteen substrate-metal pairs in columns 1 to 7.

Considering the approximations involved in the theory and the uncertainties in the assumed values of  $J$  and  $r$  one cannot expect, in general, more than an agreement in the order of magnitude between calculated and experimental values. The calculated values ( $E_c$ ) in column 9, Table III, should be evaluated on that basis. It is considered fortuitous that the calculated values other than for the alkali halides agree as well as they do with the experimental values (column 8). It is significant, however, that the highest values correspond to the substrates upon which the best oriented aluminium films were formed and that the trend definitely agrees with that characteristic

<sup>15</sup> Mayer, *J. Chem. Physics*, 1933, **1**, 270.

<sup>16</sup> Orr, *Trans. Faraday Soc.*, 1939, **35**, 1247.

of the %-orientation for all eleven substrates and with the indirectly determined substrate binding energies.

It is evident that the periodicity of the potential energy surface of the substrate-atom pair is a very important factor in defining the arrangement of the metal atoms.

TABLE IV  
DISPERSION EFFECT. ALUMINIUM ON IONIC SUBSTRATES

Substrate	Plane	Position Centre of face	Ionic Polariza- bility $\times 10^{24}$ cm. <sup>3</sup>	Charac- teristic Energy $\times 10^{12}$ ergs/ molecule	Disper- sion Constant $C \times 10^{60}$ ergs cm. <sup>6</sup>	Binding Energy $E \times 10^{-8}$ cal./ molecule
Sodium Chloride (NaCl)	(100)	Two ions	3.27	16.4	251	18
	(110)	Two ions	3.27	16.4	251	12
	(111)	Two ions	3.27	16.4	251	6
Lithium Fluoride (LiF)	(100)	Two ions	0.93	24.3	80.8	10
	(110)	Two ions	0.93	24.3	80.8	10
	(111)	Two ions	0.93	24.3	80.8	10
Mica ( $\text{KA}_2\text{Si}_2\text{O}_7(\text{OH})_2$ )	(110)	Oxygen ion	3.88	20.5	321	20
	(111)	Oxygen ion	3.88	20.5	321	22.0
	(111)	Silicon ion	0.17	2.0	3.6	2
Calcite ( $\text{CaCO}_3$ )	(1011)	Oxygen ion	3.88	20.5	321	5
Fluorite ( $\text{CaF}_2$ )	(111)	Fluorine ion	1.04	19.4	84.4	2
Zinc Blende (ZnS)	(110)	Sulphur ion	10.2	17.5	544	4
Sodalite ( $\text{Na}_4\text{Al}_3\text{Si}_3\text{O}_{12}\text{Cl}$ )	(110)	Oxygen ion	3.88	20.5	321	2

**Conclusions.**—The structure of thin aluminium films condensed in vacuum on clean ionic substrates is strongly influenced by the nature, geometry and temperature of the ions in the base. The degree of orientation of the film with respect to the base can be semi-quantitatively correlated with a binding energy characteristic of the substrate. The values of the substrate binding energy are of the same order of magnitude as van der Waals' binding between a single atom and an infinite ionic surface. The characteristics of the film structure show this method to be effective for the preparation of oxide-free oriented aluminium surfaces for studying surface reactions.

The writer is indebted to many of the research faculty for opportunities to discuss the subject and particularly to Prof. Charles Barrett, Prof. Clarence Zener and Prof. Cyril Smith of the Institute for the Study of Metals and to Prof. Joseph Mayer of the Institute for Nuclear Studies. In addition all the structure determinations were made in Prof. Barrett's X-ray diffraction laboratory by his kind permission with the assistance of Messrs. Donald Clifton and John Hess whose assistance is gratefully acknowledged.

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# THE INFLUENCE OF FOREIGN IONS ON CRYSTAL GROWTH FROM SOLUTION \*

## 1. The Stabilization of the Supersaturation of Calcium Carbonate Solutions by Anions Possessing O-P-O-P-O Chains

BY B. RAISTRICK

*Received 29th April, 1949*

An interesting case of modified crystal growth is that described by the title. When a natural water containing calcium bicarbonate is heated or made alkaline it deposits calcium carbonate as scale with the well-known undesirable consequences. Rosenstein<sup>1</sup> discovered that the addition of a few parts per million of sodium metaphosphate glass to the water (containing the equivalent of several hundred p.p.m. of calcium carbonate) would prevent this precipitation.

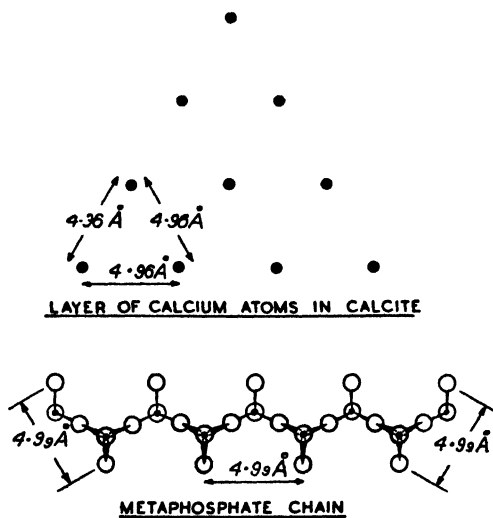


FIG. 1.

It was decided to search for an explanation of this phenomenon and one approach was to study the structures of calcite and aragonite and the probable structure of the polymetaphosphate anion. Consideration of the calcite lattice as reported by Bragg<sup>2</sup> shows that the calcium and carbonate ions are arranged in alternate layers perpendicular to the threefold axis of the lattice, and that all the calcium ions in any one layer are arranged at the corners of equilateral triangles of side length 4.96 Å (cp. Fig. 1).

<sup>1</sup> Rosenstein, *U.S. Pat.*, 2,038,316 (1936).

<sup>2</sup> Bragg, *Proc. Roy. Soc. A*, 1914, **89**, 486.

\* The substance of this paper was communicated verbally during the Discussion. It contains considerations, relevant to this Discussion, which form part of a rather broader communication to be given at the forthcoming meeting of the Canadian Institute of Chemistry at Halifax.



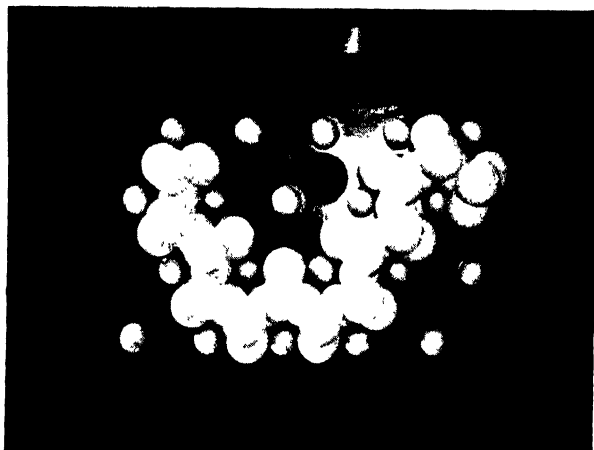


FIG. 3.- Photograph of model showing part of a metaphosphate ion adsorbed on calcite lattice.

The lower part of Fig. 1 indicates our conception of the polymetaphosphate anion. We believe that each P atom is surrounded tetrahedrally by four oxygen atoms and that these tetrahedra are linked by sharing two oxygen atoms each in such a way that a chain is obtained of the type shown. In this diagram we have assumed that the P-O-P angle is  $180^\circ$ , but a more probable angle is  $141^\circ$ ; it will be seen that the repeat distance for this anion is  $4.99 \text{ \AA}$  if we use a P-O distance of  $1.53 \text{ \AA}$  (see the papers of Levi and Peyronel,<sup>3, 4</sup> etc.). The fit between the two can be seen more clearly in Fig. 2 which shows them lying together.

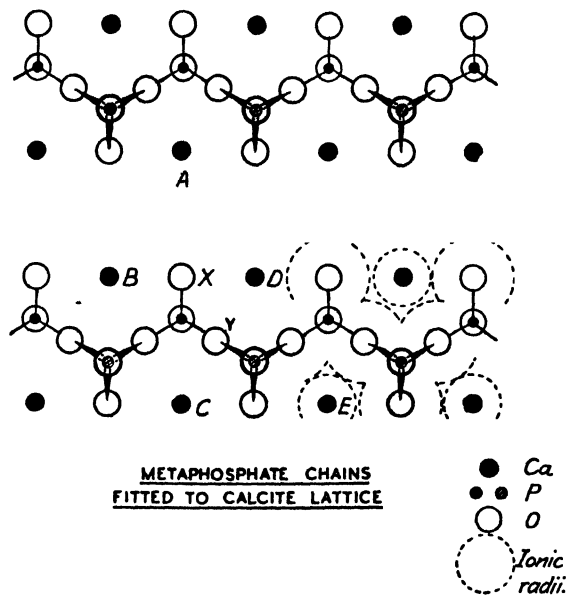


FIG. 2.

The choice of the most likely P-O-P angle in the metaphosphate chain provides an interesting problem. It will be sufficient here to say that any angle is possible from  $109^\circ 28'$  to  $180^\circ$  (excepting those which would result in steric hindrance) whilst still retaining the repeating distance of  $4.99 \text{ \AA}$  along the chain. Although  $180^\circ$  angles are not uncommon<sup>3, 4, 5, 6, 7</sup> we prefer the  $141^\circ$  angle because it permits the charge of every tetrahedral group to approach as closely as is possible to the layer of calcium ions whilst still retaining the centres of these tetrahedra immediately above the centres of the equilateral triangles formed by the calcium ions. In this case the metaphosphate chain would possess much the same structure as does the pyroxene chain found by Warren and co-workers in various metasilicates.<sup>6, 8</sup> The  $141^\circ$  angle is also a satisfactory one since the forces necessary to adjust the chain to this condition (as compared with the angle which is natural to the ion in solution) will be smaller than those necessary to cause adjustment to  $180^\circ$ , for example.

A consideration of the geometry of the calcium layer in calcite will show that it is not necessary for the metaphosphate anions to be adsorbed on the nucleus in straight chains and in fact they can twist their way about on the surface as shown in Fig. 3.

<sup>3</sup> Levi and Peyronel, *Z. Krist.*, 1935, **92**, 190.

<sup>4</sup> Peyronel, *Z. Krist.*, 1936, **94**, 311.

<sup>5</sup> Zachariasen, *Z. Krist.*, 1930, **73**, 1.

<sup>6</sup> Ito and West, *Z. Krist.*, 1932, **83**, 1.

<sup>7</sup> Machatschki, *Fortschr. Min.*, 1936, **20**, 47.

<sup>8</sup> Warren and Bragg, *Z. Krist.*, 1928, **69**, 168.

<sup>9</sup> Warren and Modell, *Z. Krist.*, 1930, **75**, 1.



Having established that the geometrical considerations are satisfactory for a hypothesis of this kind other requirements must now be considered. For example, a perfect fit might be expected to result in crystals which contain the small proportion of metaphosphate anion present as a solid solution in calcite. The explanation of the lack of tendency to form such solid solutions may be that (as can be seen from Fig. 2) each  $\text{PO}_3^-$  group is capable of replacing one  $\text{CO}_3^{2-}$  ion and if this replacement goes on over the whole of a calcium ion layer then the electrostatic potential for adsorption of another calcium ion layer will be greatly reduced. At this stage, therefore, the driving force to hold more calcium ions on to this particular surface is small and its growth will be stopped.

A further requirement of the theory is that it shall be capable of explaining the almost permanent stabilization of supersaturation, since solutions treated in this way will remain perfectly clear for weeks. Inspection of the lattice shows that metaphosphate can adsorb equally effectively on the top of one calcium layer of a nucleus and on the bottom of another calcium layer of the same nucleus. In this way the thickness of the nucleus can be held at embryonic dimensions in such a way that the embryo does not become big enough to be incapable of dissociating completely once more into disordered ions. In this connection the point may be made that the high effective curvature at the perimeter of an extremely thin layer structure will militate against growth on this perimeter.

It is interesting that nitrate and iodate ions which have about the correct size and shape for being adsorbed on the lattice, and also carry the correct charge for leaving the calcite surface electrically neutral, show little or no stabilizing effect. This is probably because, owing to their single charge, they are competing for the surface on terms which put them at a disadvantage as compared with the carbonate ion. The metaphosphate ion, on the other hand, with its spaced-out multiplicity of single charges will be electrostatically at a considerable advantage compared with a carbonate ion, whilst in respect of the difference between the entropy in the dissolved state and the entropy in the adsorbed state it is not at any serious disadvantage. It is probably the possession of this multiple charge which accounts for the reported ability of the long-chain metaphosphate ion to stabilize supersaturated solutions of a large number of substances. These other cases normally require higher concentrations of metaphosphate and the effects are not usually so clean-cut as in the case under consideration. They are probably due to a somewhat grosser effect than the one that we are discussing here with its special geometrical aspect and possibility of leaving the embryo surface in a condition of electrical neutrality.

Reitemeier and Buehrer<sup>10</sup> investigated the stabilization of calcium carbonate solutions very thoroughly in the laboratory and show some excellent photomicrographs of the distorted calcite crystals which result when metaphosphate or pyrophosphate are used in amounts that are insufficient to effect permanent stabilization. This distortion is probably due to the dislocations caused in the lattice by a limited number of these foreign metaphosphate ions which are capable of a two-dimensional fit, as described, but do not fit in the third dimension.

Our hypothesis to explain the stabilization of supersaturation is as described above and any chain-like anion consisting of repeating phosphorus-oxygen bonds should be capable of effecting this stabilization. We have confirmed the observations of Reitemeier and Buehrer on the effectiveness of 2 p.p.m. of sodium metaphosphate glass or sodium pyrophosphate in stabilizing the supersaturation, and have also shown that sodium tripolyphosphate (triphosphate) is equally effective, whilst the fibrous sodium metaphosphate ( $P_{2.1/n}$ ,  $a = 7.60 \text{ \AA}$ ,  $b = 6.02 \text{ \AA}$ ,  $c = 11.32 \text{ \AA}$ ,  $\beta = 93^\circ 58'$ ) is effective at several times the concentration. For reasons which cannot adequately be given here we believe that all these materials possess the repeating O-P-O bonds which are necessary on the basis of the theory and that they only differ by virtue of the number of phosphorus atoms in the chain.\*

<sup>10</sup> Reitemeier and Buehrer, *J. Physic. Chem.*, 1940, **44**, 535 and 552.

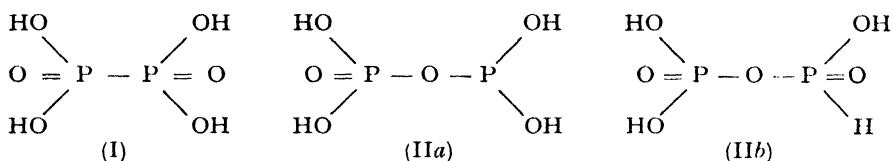
\* It is realized that the proportion of end-groupings is highest with the short chain compounds and that the approximation to electrical neutrality may not be so close as is possible with the chain metaphosphate ion.

Similarly we would expect the following classes of compound to be ineffective, and we have confirmed this to be true.

(i) THE SOLUBLE TRI- AND TETRAMETAPHOSPHATES.—These we believe<sup>11</sup> to contain cyclic anions consisting of six- and eight-membered rings respectively. These compounds possess the essential repeating O-P-O bonds, but their more rigid cyclic structures prevent adaptation to the calcite lattice. It is interesting that the ineffective trimetaphosphate ring can be split open in quantitative fashion by hydrolysis to produce the chain-like tripolyphosphate, which, as already stated, is very effective.

(ii) THE METASILICATES.—The increased length of the Si-O bond as compared with the length of the P-O bond results in a repeating distance<sup>8,9</sup> for the metasilicate chain of 5.24 Å as compared with that of about 4.99 Å proposed for the metaphosphates when adsorbed on the calcite lattice. Thus, even if despite considerable hydrolysis the metasilicate chain structure does to some extent persist into aqueous solution, there could not be a fit with the calcite lattice in the same way as is possible with metaphosphate. Actually, with sodium metasilicate in concentrations from 2 to 170 p.p.m. there is no appreciable stabilization of a supersaturated solution containing the equivalent of 200 p.p.m. of calcium carbonate.

(iii) COMPOUNDS CONTAINING P-O BONDS IN THE INCORRECT SEQUENCE.—The only two compounds that we have studied in this class are sodium hypophosphate  $\text{Na}_4\text{P}_2\text{O}_6$  and sodium perdisphosphate  $\text{Na}_4\text{P}_2\text{O}_8$ . Regarding the hypophosphate anion there have been two main schools of thought on its structure. The choice lies between (I) and (IIa) or (IIb) for the structure of the acid.



On the basis of our hypothesis a compound with the structure (II) should be capable of stabilizing the supersaturation whilst (I) should be ineffective. We believe that the structure is (I) for reasons already advanced<sup>12</sup> and certainly sodium hypophosphate shows little or no ability to stabilize the supersaturation.

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<sup>11</sup> Unpublished X-ray and other observations.

<sup>12</sup> Raistrick and Hobbs, *Nature* (in press).

## ORIENTED OVERGROWTHS AND STABILIZATION AT ORDINARY TEMPERATURES OF THE CUBIC (I), TETRAGONAL (II) AND ORTHORHOMBIC (III) PHASES OF AMMONIUM NITRATE

BY RAYMOND HOCART AND Mlle. AGNÈS MATHIEU-SICAUD

*Received 9th February, 1949*

The experiments described in this paper fall into two groups. Firstly, a study has been made on the polarizing microscope of oriented overgrowths of the various polymorphic forms of ammonium nitrate on muscovite mica. The development of fissures resulting from the change of one polymorphic form into another provides an indication of the degree of correspondence,

as regards densely packed atomic planes, between one form and another, in agreement with the paramorphism lattice theory first developed by Friedel.<sup>1</sup> Secondly, experiments have been made on the stabilization at ordinary temperatures of ammonium nitrate I, II and III by the addition of impurities possessing lattice constants close to those of a particular polymorphic form of  $\text{NH}_4\text{NO}_3$ .

### Experimental

**Oriented overgrowths on mica.**—Oriented overgrowths were formed between cleavage surfaces of mica, either by melting a small fragment of the salt or from a supersaturated solution. An isotropic support for the oriented nitrate crystals was made from a rectangular sheet of mica with edges approximately parallel and perpendicular to  $n_g$  cut in the manner shown in Fig. 1. By placing portions of the upper and lower sheets in the crossed positions at *a* and *b* the path differences due to the mica are eliminated, and the oriented overgrowths could be examined microscopically between crossed Nicols.

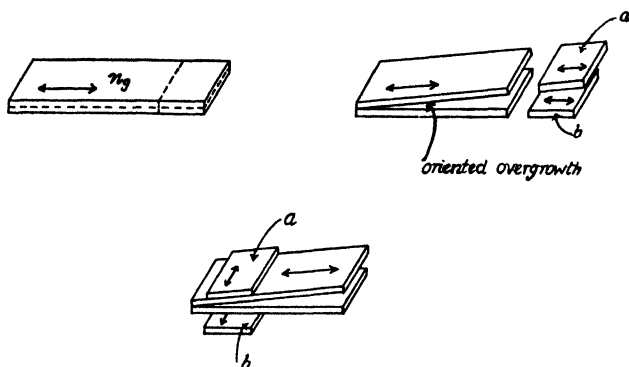


FIG. 1.

(a) PHASE I ( $[100] = 4.40 \text{ \AA}$ )<sup>2</sup>

Lattice constants of mica (001) plane cell are

$$[100] = 5.18 \text{ \AA}; [010] = 9.02 \text{ \AA}.$$

Superposition trials of (001) mica lattice and (001) nitrate I lattice lead one to expect two simple coincidences of plane cells.

In the first one (Fig. 2 I a), two square (001) nitrate I cells fall in line along  $[010]$  mica, over the rectangular (001)-centred cell. The computed approximation is  $-15\%$  for  $4.40 \text{ \AA}$  nitrate in comparison with  $5.18 \text{ \AA}$  mica and  $-2.4\%$  for  $2 \times 4.40 \text{ \AA}$  nitrate in comparison with  $9.02 \text{ \AA}$  mica. In the second coincidence (Fig. 2, I b), four square (001) nitrate cells, making a square pattern of sides  $2 \times 4.40 \text{ \AA}$  nearly cover a pseudo-square (multiple of 4) cell of mica built on  $[110] = 10.40 \text{ \AA}$  and  $[3\bar{1}0] = 8.98 \text{ \AA}$  (and are symmetrical with respect to the mica (001) symmetry plane). Approximation calculations give  $-2\%$  for  $2 \times 4.40 = 8.80 \text{ \AA}$  nitrate in comparison with  $8.98 \text{ \AA}$  mica and  $-15.4\%$  for  $8.80 \text{ \AA}$  nitrate as compared with  $10.40 \text{ \AA}$  mica. The better coincidence along  $[3\bar{1}0]$  mica should lead to extended overgrowths along that row, that is to say, inclined at about  $60^\circ$  to  $[010]$  mica.

<sup>1</sup> Friedel, *Leçons de Cristallographie* (Paris—Nancy, 1926).

<sup>2</sup> Hendricks, Posnjak and Kracek, *J. Amer. Chem. Soc.*, 1932, **54**, 2765.

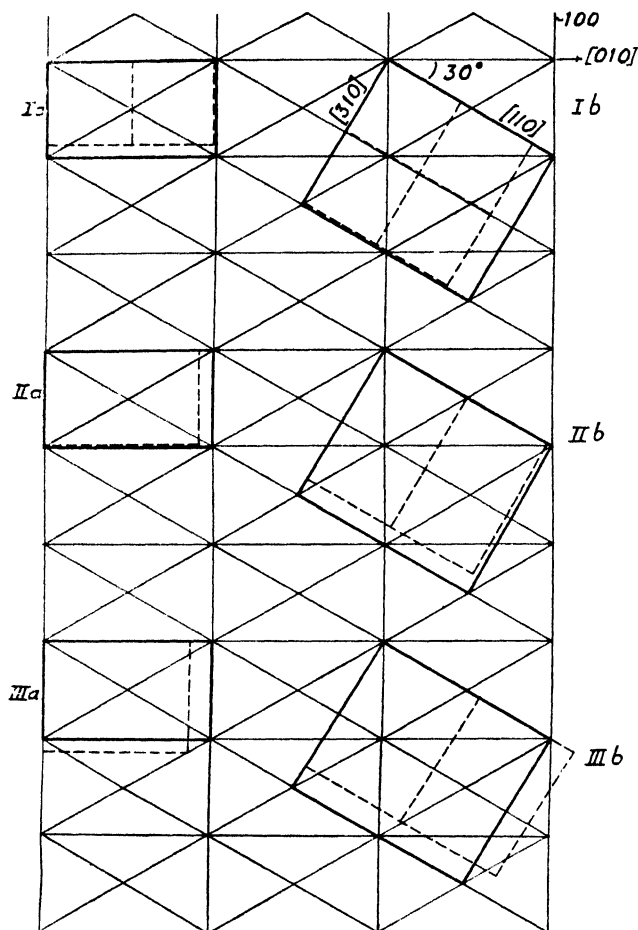


FIG. 2.—Pattern of ammonium nitrate phases I, II, III on mica.

## (b) PHASES II, III and IV

Patterns of analogous type are valid for phases II, III and IV (Fig. 2, II *a*, *b*, III *a*, *b*), reticular planes (110) for II, (100) for III, (110) for IV, growing over (001) mica.

For brevity and clarity, only approximate coincidences with [110] and  $[3\bar{1}0]$  mica are reported below, for comparisons.

PHASE I  $[100]$ :  $4.40 \times 2 \text{ \AA}$  : - 2 % relative to  $[3\bar{1}0]$  =  $8.98 \text{ \AA}$  mica;  
 $[010]$ :  $4.40 \times 2 \text{ \AA}$  : - 15 % relative to  $[110]$  =  $10.40 \text{ \AA}$  mica.

PHASE II  $[110]$ :  $8.13 \text{ \AA}$  : - 9.5 % relative to  $[3\bar{1}0]$  mica;  
 $[001]$ :  $5 \times 2 \text{ \AA}$  : - 4 % relative to  $[110]$ .

PHASE III  $[010]$ :  $7.66 \text{ \AA}$  : - 15 % relative to  $[3\bar{1}0]$ ;  
 $[001]$ :  $5.80 \times 2 \text{ \AA}$  : + 11.5 % relative to  $[110]$ .

(c) By melting and cooling  $\text{NH}_4\text{NO}_3$ , overgrowths are usually oriented at  $30^\circ$  and  $60^\circ$  to  $[010]$  mica, with predominance of the latter orientation

(Fig. 3, 4). Also, in agreement with alterations of lattice constants between successive phases, fissures observed in homogeneous areas are more numerous at  $30^\circ$  from  $[010]$  mica than at  $60^\circ$  when phase I  $\rightarrow$  phase II (Fig. 2, 5). When phase II  $\rightarrow$  phase III, fissures at  $30^\circ$  from  $[010]$  are seen to widen, while those at  $60^\circ$  become narrower and appear as lines (Fig. 2, 6, 7). On the other hand, when phase II gives place directly to IV, the lattice constants are more similar than the cases II, or IV, with III; here, any change in width of fractures is hardly detectable.

These observations are consistent with the law of mutual correspondence between dense lattice planes when a paramorphic transformation takes place.

Overgrowths oriented at  $0^\circ$  and  $90^\circ$  from  $[010]$  mica, however plausible for  $\text{NH}_4\text{NO}_3$  I, only appear when phase IV undergoes recrystallization within a thick<sup>3</sup> and not too dry section. In this case, IV becomes independent of I and the orientations just cited appear in I simultaneously with overgrowths at  $30^\circ$  and  $60^\circ$  and with about the same frequency. During the heating process, those two types of overgrowths remain in phases III, II, I. Such a "pilot action," either of I or of IV, is in accord with the interdependence of paramorphic phases in a given substance.

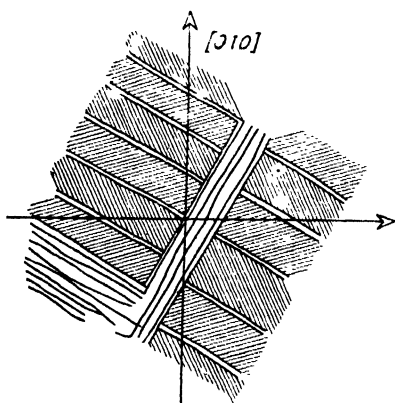


FIG. 3.

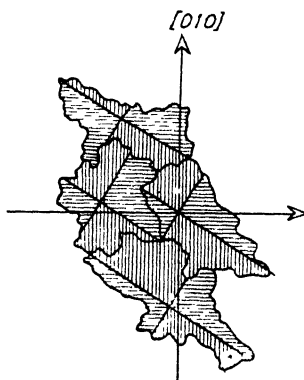


FIG. 4.

Ammonium nitrate — oriented overgrowths.

**Stabilization.**—In order to study individual phases at ordinary temperature we endeavoured to stabilize them by incorporation of a small quantity of suitable impurities. The essential idea was to add to  $\text{NH}_4\text{NO}_3$  crystalline substances having two (or three, or even only one) lattice constants closely similar to those of the phase considered in order to have a dense plane cell in concordance. Tests were made either by fusion or by solution with about 1 to 2 % of impurity added to the  $\text{NH}_4\text{NO}_3$ . Stabilized phases, when obtained, can be preserved unchanged indefinitely, provided that they are protected from moisture, since the hygroscopic character of  $\text{NH}_4\text{NO}_3$  destroys any stabilization.

Among the impurities employed, some give overgrowths by themselves upon mica, others do not. A salt capable of stabilizing one of the forms of  $\text{NH}_4\text{NO}_3$  need not itself form oriented overgrowths on mica. For example,  $\text{PbCl}_2$  gives oriented overgrowths,  $\text{KMnO}_4$  does not; each of them stabilizes phase III.

<sup>3</sup> Bowen, *J. Physic. Chem.*, 1926, 30, 721.



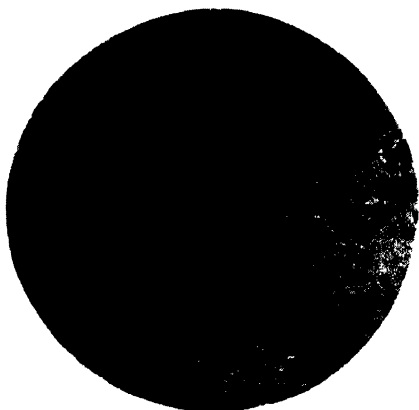


FIG. 8.—Phase II stabilized with  $\text{PbCO}_3$ .

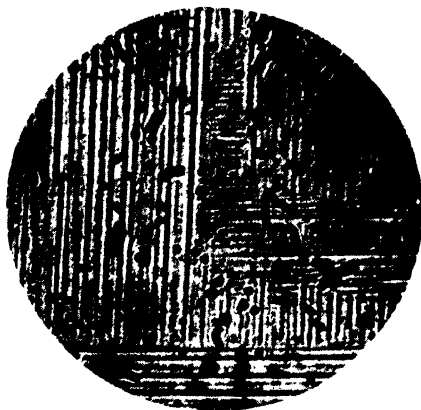


FIG. 9.—Phase III stabilized with  $\text{KMnO}_4$ .

PHASE I ( $a_0 = 4.40 \text{ \AA}$ )

Stabilizers : NaF (cubic),  $a_0 = 4.62 \text{ \AA}$  ; relative deviation in comparison with nitrate, 5 %.

$\text{NH}_4\text{Br}$ ,  $a_0 = 4.04 \text{ \AA}$  ;  $\text{NH}_4\text{Cl}$ ,  $a_0 = 3.86 \text{ \AA}$  ; the last substance only delays the transformation I  $\rightarrow$  II.

$\text{NH}_4\text{I}$ ,  $a_0 = 7.24 \text{ \AA}$  is without effect owing to the large discrepancy between lattice constants.

$\text{RbIO}_3$ ,  $a_0 = 4.52 \text{ \AA}$  ;  $\text{NH}_4\text{IO}_3$ ,  $a_0 = 4.51 \text{ \AA}$ .

In all these examples, overgrowths of  $\text{NH}_4\text{NO}_3$  are oriented at  $30^\circ$  and  $60^\circ$  from  $[010]$  mica.

With  $\text{KIO}_3$  in solution ( $a_0 = 4.46 \text{ \AA}$ ), the two kinds of oriented overgrowths expected appear at  $30^\circ$  and  $60^\circ$ , and at  $0^\circ$  and  $90^\circ$ , from  $[010]$  mica. It has been stated above that this last series does not appear when the nitrate alone is cooled from the molten state but only after recrystallization of phase IV.

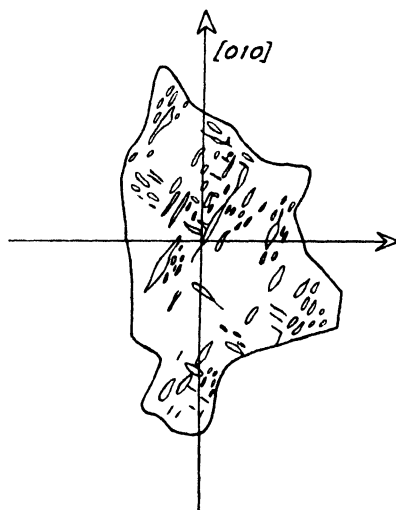


FIG. 5.—Fissures in oriented overgrowths.

PHASE II ( $a_0 = 5.75$ ,  $c_0 = 5$ )

$\text{PbCO}_3$  (Fig. 8) is a good stabilizer (orthorhombic):  $a_0 = 8.47 \text{ \AA}$  ;  $b_0 = 6.14 \text{ \AA}$  ;  $c_0 = 5.16 \text{ \AA}$ . Relative deviations : + 4.2 % for  $8.47 \text{ \AA}$  compared with  $[110] = 8.13 \text{ \AA}$  nitrate ; + 3.2 % for  $5.16 \text{ \AA}$  compared with  $[001] = 5 \text{ \AA}$  nitrate ; it gives the two predicted oriented series,  $30^\circ$  ( $60^\circ$ ) and  $0^\circ$  ( $90^\circ$ ) which are also produced with  $\text{CsNO}_3$  (previously assigned as stabilizer by Wallerant <sup>4</sup>) (hexagonal) :  $a_0 = 10.74$  ;  $c_0 = 7.68$ .

$\text{NaNO}_2$  (orthorhombic) :  $a_0 = 3.55 \text{ \AA}$  ;  $b_0 = 5.56 \text{ \AA}$  ;  $c_0 = 5.37 \text{ \AA}$

$\text{Ag}_2\text{SO}_4$  (orthorhombic) :  $a_0 = 5.82 \text{ \AA}$  ;  $b_0 = 12.65 \text{ \AA}$  ;  $c_0 = 10.25 \text{ \AA}$  .

$\text{KClO}_4$  (orthorhombic) :  $a_0 = 8.85 \text{ \AA}$  ;  $b_0 = 5.65 \text{ \AA}$  ;  $c_0 = 7.23 \text{ \AA}$

$\text{Cs}_2\text{SO}_4$  (orthorhombic) :  $a_0 = 6.34 \text{ \AA}$  ;  $b_0 = 10.92 \text{ \AA}$  ;  $c_0 = 8.22 \text{ \AA}$ .

<sup>4</sup> Wallerant, *Bull. Soc. Miner. France*, 1905, **28**, 311.



## OVERGROWTHS OF AMMONIUM NITRATE

PHASE III ( $a_0 = 7.06 \text{ \AA}$ ;  $b_0 = 7.66 \text{ \AA}$ ;  $c_0 = 5.80 \text{ \AA}$ )

$(\text{NH}_4)_2\text{SO}_4$  (orthorhombic):  $a_0 = 5.95 \text{ \AA}$ ;  $b_0 = 10.56 \text{ \AA}$ ;  $c_0 = 7.73 \text{ \AA}$ ; is a good stabilizer but restricts the number of oriented overgrowths belonging to the two series.

$\text{KMnO}_4$  (orthorhombic):  $a_0 = 9.10 \text{ \AA}$ ;  $b_0 = 5.60 \text{ \AA}$ ;  $c_0 = 7.40 \text{ \AA}$

$\text{KClO}_3$  (monoclinic):  $a_0 = 4.65 \text{ \AA}$ ;  $b_0 = 5.58 \text{ \AA}$ ;  $c_0 = 7.08 \text{ \AA}$

$\text{NaClO}_4$  (orthorhombic):  $a_0 = 6.48 \text{ \AA}$ ;  $b_0 = 7.06 \text{ \AA}$ ;  $c_0 = 7.08 \text{ \AA}$

$\text{KI}$  (cubic):  $a_0 = 7.05 \text{ \AA}$

$\text{Rb}_2\text{SO}_4$  (orthorhombic):  $a_0 = 5.95 \text{ \AA}$ ;  $b_0 = 10.39 \text{ \AA}$ ;  $c_0 = 7.78 \text{ \AA}$ ; efficiency is poorer with  $\text{KF}$  (cubic):  $a_0 = 5.33 \text{ \AA}$ , and  $\text{PbCl}_2$  (orthorhombic):  $a_0 = 4.52 \text{ \AA}$ ;  $b_0 = 7.61 \text{ \AA}$ ;  $c_0 = 9.03 \text{ \AA}$ ; with this last substance the relative approximation for  $7.61 \text{ \AA}$  compared with  $7.66 \text{ \AA}$  is  $0.7 \%$  but as much as  $22 \%$  for  $4.52 \text{ \AA}$  compared with  $5.80 \text{ \AA}$ .

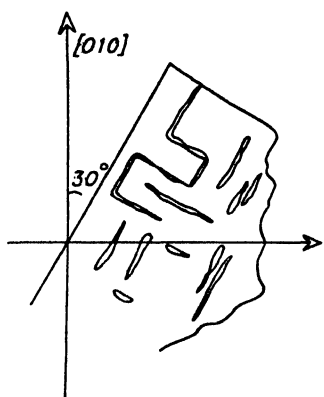


FIG. 6.

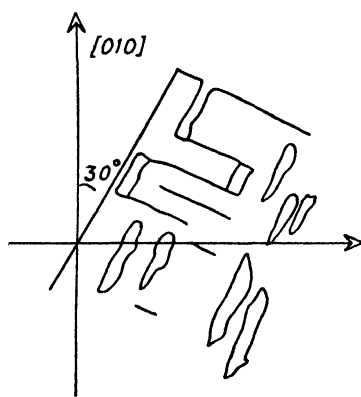


FIG. 7.

Alterations in fissures connected with change II→III.

To sum up, all facts reported here show that stabilization does not seem to depend upon either the aptitude of the stabilizer to give oriented overgrowths by itself, or on chemical analogy between impurity and  $\text{NH}_4\text{NO}_3$ . Moreover, impurity and nitrate do not show any symmetry relationship regarding space lattices, but stabilization is frequently obtained when two sets of dense plane cells mutually coincide dimensionally to within the limits usually accepted in oriented overgrowths.

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# HABIT MODIFICATION IN CRYSTALS AS A RESULT OF THE INTRODUCTION OF IMPURITIES DURING GROWTH

BY H. E. BUCKLEY

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It was the writer's lot, some quarter of a century ago, to become interested in problems connected with the growth of crystals and particularly in that aspect which is embodied in the title of this article.

During this period, ideas have been introduced and views expressed regarding the mechanism of habit change and comments made on the efforts of the few other workers who have been attracted to this subject. Some of the various views on the means whereby a crystal changes its habit will be dealt with in the present paper, though it is the writer's opinion that up to the present time (February, 1949) no satisfactory explanation has yet been forthcoming.

However, now that the popularity of this field appears to have increased and attracted the interest of many workers who bring their skill, developed in other directions, to the problem we shall not be long in reporting substantial progress and possibly reaching satisfactory conclusions. For those who have not been able to devote lengthy periods to a study of the different aspects of the subject, the present brief account of the various difficulties which are inseparable from each explanation of the results may be helpful, and possibly informative.

It should be pointed out at once that many views expressed about crystal growth and habit change are inadequate on account of the few facts upon which they have been based. The idea that relative solubilities of crystal and impurity can have any bearing on the result is one of these.

**Types of Impurity.** The vast proportion of work on the subject has been performed with aqueous solutions, the only well-marked habit change noted for another type of liquid being that of Jenkins<sup>1</sup> who reported the extension of the {110} planes on naphthalene crystals grown from methanol in the presence of collodion. Wells<sup>2</sup> has given several instances of change of crystal habit by varying the solvent itself, but this is not dealt with in the present paper, though the solvent cannot be entirely disregarded as a factor in inducing a crystal to clothe itself with certain surfaces rather than with others. In aqueous solution, then, we have the ions of the dissolved crystals (if ionized) and possibly undissociated molecules. We have minute concentrations of H<sup>+</sup> and OH<sup>-</sup> ions; concentrations which may be increased to formidable proportions when acid or alkali is added to the solution or where the crystallizing material itself provides them, e.g., borax, K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub>, alum, etc. Whenever a soluble impurity is added to the saturated solution of the substance several things may happen. (a) The impurity may have one common ion, e.g., with K<sub>2</sub>S<sub>2</sub>O<sub>3</sub> added to K<sub>2</sub>SO<sub>4</sub>. In this case, only one new ion is added to the solution. (b) There may be no common ion, e.g., when Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> is added to K<sub>2</sub>SO<sub>4</sub>. In this event, if double decomposition does not occur at once, it may possibly happen when the solution becomes supersaturated by evaporation or cooling. Thus, when Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> is added in substantial amounts to K<sub>2</sub>SO<sub>4</sub> solution, the four immediately obvious

<sup>1</sup> Jenkins, *J. Amer. Chem. Soc.*, 1925, **47**, 903.

<sup>2</sup> Wells, *Phil. Mag.*, 1946, **37** (7), 184, 217.

possibilities are  $K_2SO_4$ ,  $Na_2SO_4$ ,  $K_2S_2O_8$ ,  $Na_2S_2O_8$ . The latter two salts are very soluble in their hydrated form and would not be expected to come down before the others. Actually, a double salt,  $NaK_8(SO_4)_2$  crystallizes in these circumstances. There is, here, no *complex ion* formed, but this also is a possibility, sometimes found, viz. :—

(c) Where the impurity can join with the salt to form another salt containing a complex ion, which will also be present in the solution. A good example of this is when  $PbCl_2$  is added to  $KCl$ , the compound  $KPbCl_3$  being formed.<sup>3</sup> Finally we have an added complication when the molecule is very big.

(d) Thus large basic or acidic dye molecules which dissociate to form a simple ion, e.g.,  $Na^+$  or  $Cl^-$  and a huge residue, anion or cation as the case may be. They are only a special case of (a) or (b) above. But whereas, when dealing with an "impurity" like  $Na_2SO_4$ , we have a substance which will itself *readily* crystallize when given the opportunity, the acid dyes in particular are poor crystallizers, so much so that when they do happen to possess this property to a noticeable degree they usually show it in their names, e.g., Crystal Scarlet, Crystal Ponceau, etc. (mostly poor habit modifiers). It is hardly likely, then, than that they will go so contrary to their usual behaviour as to crystallize in small units in a "host" crystal when they are present to perhaps only one thousandth part of their own saturation limit. This is what one view would, however, lead us to accept (see later).

### Phenomena often associated together during the Growth of a Crystal.

I. Deposition of small crystals of the impurity upon some plane, or set of equivalent planes, so that some zone axis of the one is parallel to a zone axis of the other.

A face of the "impurity" crystal is parallel to some face of the crystal though the latter is not always developed. A good example<sup>4</sup> is  $KMnO_4$  on  $KClO_3$  where the  $y$ -axes of the two coincide (Plate I) and the (100) face of the small  $KMnO_4$  crystals is parallel to the (100) face, frequently absent, of  $KClO_3$ . The large basal plane (001) of  $KClO_3$  which is a prominent feature of these crystals has nothing in the  $KMnO_4$  crystals parallel to it.

It is necessary for this phenomenon to occur that both "crystallizing substance" and "impurity" are supersaturated. That it is not too intimately related to habit change is seen from the fact that the  $KClO_3$  plates, on which the  $KMnO_4$  crystals grow in parallel position, show practically no change of habit. Yet some such mechanism was adopted by Gaubert<sup>5</sup> to explain the growth of {100} on lead and barium nitrate crystals in the presence of the basic dye Methylene Blue (*Colour Index*, No. 922). And in this case the dye certainly was deposited thickly on the cube planes while the adjacent octahedron planes were quite free. Gaubert introduced the term "syncrystallization" to cover this type of mutual arrangement. In spite of this, crystals of barium nitrate with no trace of the dye and yet modified to cubes were noted by Walcott.<sup>6</sup> Other examples where two substances could act as "habit-modifying impurities" towards each other have been given by Bunn,<sup>7</sup> e.g., in ammonium chloride or bromide and urea. This author first emphasized the view, later endorsed by Royer<sup>8</sup> and by

<sup>3</sup> Mehmel and Nespital, *Z. Krist. A*, 1934, **88**, 345.

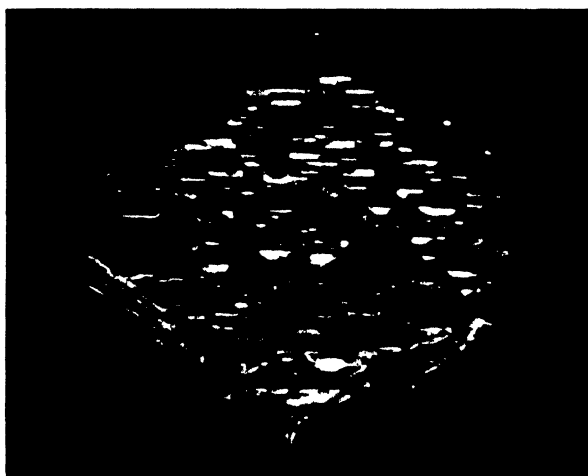
<sup>4</sup> Buckley, *Z. Krist. A*, 1932, **82**, 46; *ibid.*, 1937, **97**, 370.

<sup>5</sup> Gaubert, *Rev. Sci.*, 1910, **48**, 74.

<sup>6</sup> Walcott, *Amer. Miner.*, 1926, **2**, 221, 259.

<sup>7</sup> Bunn, *Proc. Roy. Soc. A*, 1933, **141**, 567.

<sup>8</sup> Royer, *Compt. rend.*, 1935, **198**, 185.



1. Parallel growth of  $\text{KMnO}_4$  crystals on a plate of  $\text{KClO}_3$ . The  $[010]$  directions in each are parallel.



Frondel,<sup>9</sup> that for a pair of substances to act in this manner, some one surface of the one crystal should have a regular repetition of atom-pattern approximating sufficiently to some plane in the other substance (when crystallized) and that cations and anions in the two should be similarly arranged (along a row at least) and at similar intervals. That these planes where such coincidences are to be sought are not always planes of lowest indices, such as are almost invariably found on the finished crystals (vicinal planes apart), is seen from the work of Mehmehl and Nespital<sup>8</sup> where, in the parallel growths of layers of KCl and KPbCl<sub>3</sub>, such planes as (121) and (323) are worked out as being in contact with the (111)-KCl. Before discussing the necessity for any such conditions to apply to substances able to modify habit, we must review another method of inclusion of impurity.

## II. Orientated inclusion of impurity on an ionic or molecular scale.

This kind of inclusion was first emphasized by the writer in his papers of 1930-34 where it was suggested that ionic adhesion was likely to be by *single* ions strewn about the adsorbing surface in a statistically even manner, and as attachment would be the same for each ion, the latter would be orientated all one way. His views at the time were that this was the process by which modification of the crystal habit would occur rather than by the more severely restricted mode of parallel deposition of whole (if small) crystals.

The same mode of attachment to the growing surface and consequent modification of habit was accepted by Frondel<sup>9</sup> and France<sup>10</sup> though the former accepted Bunn's and Royer's viewpoint as well. Gaubert, too, extended his views on habit change to include both these types of interference with the surface, i.e., the earlier ones being "syncrystallizations" and the present ones solid-solutions. The actual outward appearance of a crystal which has included coloured impurity in this manner is well known, there being many examples afforded among natural minerals, and artificial examples are easily prepared. They are of two sorts: (a) inclusions in layers parallel to certain faces, the colour often filling the crystal or large portions of it, and (b) inclusions in well-marked zones, stretching from some point near the centre of the crystal to certain faces, while avoiding others. If a crystal of alum or lead nitrate *started* as a small cube-seed and coloured impurity was uniformly deposited on the *cube* faces all along, the colour would necessarily be distributed *throughout* the crystal. If the deposition on *cube* faces commenced, using an *octahedral* seed, coloured and colourless portions would certainly be segregated. Where the symmetry is sufficiently low, hour-glasses are the result; with higher symmetry, more planes can co-operate and threefold "propellers," fourfold "Maltese crosses" and so on may result. The coloured substance usually shows the optical phenomenon called pleochroism, i.e., change of tint in the coloured parts of the crystal as the azimuth of a single polarizing prism or plate is changed. The conditions for this pleochroic change in colouring matter included in crystals, and why it is occasionally found even in isotropic cubic crystals, were discussed in a paper by the writer.<sup>11</sup> It should be noted, however, that the "hour-glass" may be only a feature *accompanying* the mode of crystal deposition as crystals, e.g., of NH<sub>4</sub>ClO<sub>4</sub> of quite *unmodified habit* have been observed at times to develop colourless hour-glasses containing tiny inclusions of mother-liquor. Without getting down to molecular dimensions, it is impossible to say whether the impurity hour-glasses are made up of tiny crystallites in parallel formation or are as described by the writer, but it

<sup>9</sup> Frondel, *Amer. J. Sci.*, 1935, **30** (5), 51.

<sup>10</sup> France, (*inter al.*) *Alexander's Colloid Chemistry*, Vol 5 (1944), 443.

<sup>11</sup> Buckley, *Mem. Proc. Manchester Lit. Phil. Soc.*, 1939, **83**, 51.

does appear extremely improbable that crystallites will form on a crystal surface under conditions of gross undersaturation of the impurity. Nor does the explanation—that cation-anion distances are a necessary feature of habit change, as they are of parallel-growth formation—appear feasible when we consider such widely divergent types of impurity as potassium chromate and the huge dye-molecule Brilliant Croceine 9B (*Colour Index*, No. 313), both of which affect the  $\{011\}$  faces of  $\text{KClO}_3$  crystals. The relationships of both modes of attachment to a crystal surface (with subsequent incorporation) to habit modification will be dealt with later.

In the meantime, one further growth-phenomenon will be briefly discussed.

**Inner Dendrites in Crystals.** It is impossible to cover adequately in a short paper all that is known of the formation and occurrence of dendritic crystals. What is of interest here is that crystals, whether modified or not, often show a dendritic interior which has later been filled in by slow uniform growth. Has the shape of the original dendrite anything to do with the final habit of the crystal?

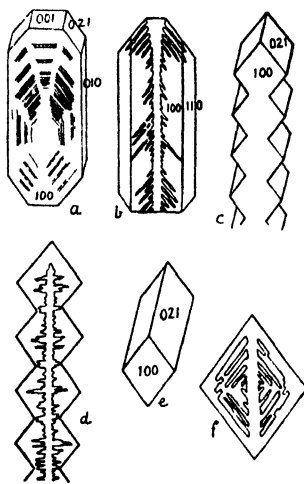


FIG. 1.—Potassium hydrogen oxalate; different dendrite plans: (a) ordinary; (b) with Acridine Orange N.O.; (c) and (d) with Orseilline BB; (e) and (f) with Brilliant Azurine B.

In some cases, the original dendrite is different in the pure crystals and in those modified by various impurities and it is only to be expected that this avenue of possible explanation of habit change must be entered. Acid potassium oxalate is modified in several ways. The pure habit has  $\{100\}$ ,  $\{010\}$  and  $\{001\}$  well developed (Fig. 1 a). In the presence of Acridine Orange N.O. (*Colour Index*, No. 788), thin flats on  $\{100\}$  bevelled by  $\{110\}$  and elongated on the  $z$ -axis are formed. Here,  $[001]$  is the direction of the *main stem* of the inner dendrite and  $[01\bar{2}]$ , i.e., edge  $(021) : (100)$ , that of the first or primary branching. The secondary branchings parallel to  $[100]$  are only vestigial (Fig. 1 b). A different habit is developed by Orseilline BB (*Colour Index*, No. 284). With  $\{100\}$  and  $\{021\}$  predominating, the dendrite has the same  $[001]$  main stem, there being elongation on  $z$  and corugated sides due to oscillatory repetition of  $(021)$  and  $(02\bar{1})$  (Fig. 1 c). But  $[010]$  is the primary branching direction, followed by  $[100]$  (Fig. 1 d). An identical habit change, except that elongation is on  $[100]$ , is afforded

by the crystals grown with Brilliant Azurine B (*Colour Index*, No. 511) and some other impurities (Fig. 1 e). Again,  $\{100\}$  and  $\{021\}$  are developed, but here  $[100]$  is the main stem,  $[001]$  the primary branching and  $[01\bar{2}]$  the secondary (Fig. 1 f).

In our present state of knowledge it is not possible to estimate what the significance of these varying inner-dendrite patterns may be.

III. The most important phenomenon associated with the growth of a crystal is, in this article at least, the capacity of certain impurities to modify the habit of the crystals from that developed during pure growth. This feature is now well known and an effort will be made here to assess the value of various viewpoints as to the causes thereof. As a preliminary we may advance a few figures to show how little influence the relative solubilities

of crystal and impurities have upon the habit. During the course of twenty-five years' observation of these phenomena and inspection of the results of over 12,500 separate crystallizations of thirty odd crystalline materials, solubility does not appear to have any bearing on habit change except the obvious and indirect one that if an impurity is sufficiently salted-out by the crystal solution, it cannot be expected to produce much habit change. Even so, if a trace remains in solution it may yet have a stronger influence on the habit than some other—far more soluble—impurity.

TABLE I

SOLUBILITY, AND EFFECTIVENESS IN INDUCING HABIT CHANGE ON  $\text{KClO}_3$  — {011}

Impurity	$\text{S}_2\text{O}_3^{2-}$ ion	$\text{CrO}_4^{2-}$ ion	Eosamine (Colour Index, No. 119)	Sulphon Black (Colour Index, No. 271)	Trypan Red (Colour Index, No. 438)
Solubility in saturated $\text{KClO}_3$ solution	Very soluble	Very soluble	Soluble	Rather insoluble	Soluble
Effectiveness	>0	(011) > (001) at 1 in 3	(011) > (001) at 1 in 100	(011) > (001) at 1 in 10,000	(011) > (001) at 1 in 60,000

Table I (which could be considerably lengthened) gives some idea of the lack of correlation between modifying power and solubility.

It is now time to weigh up the merits of the two kinds of attachment proposed at various times by Bunn and Royer on the one hand and the writer on the other, viz., whether attachment is by several ions together to form a minute crystal possessing its own (if distorted) space lattice in the host crystal or as isolated ions strewed here and there but possessing a similar orientation. In the former case, cation-anion distances are important and

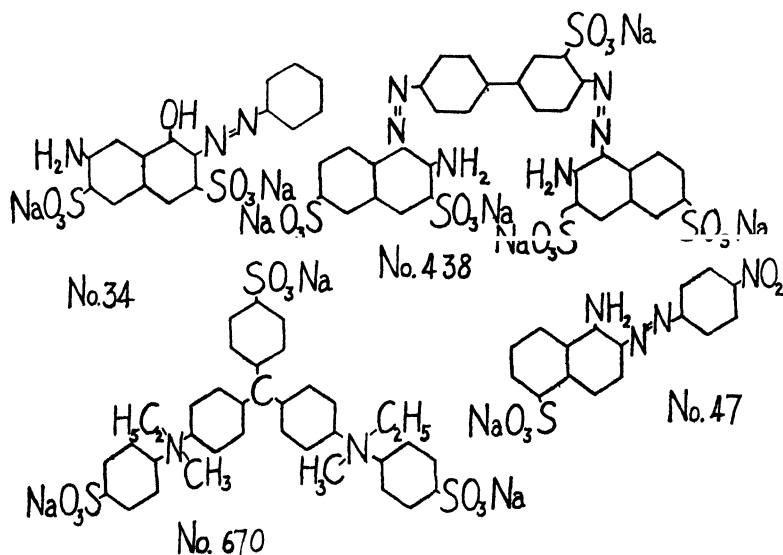


FIG. 2.—Configurations of four impurities which have an identical effect on  $\text{K}_2\text{CrO}_4/010$ .



some correlation is to be expected. That this does happen at times has been shown in the quoted works of Bunn, Frondel and others. But in nearly all these cases, the habit modification is moderate and only attained with great concentrations of impurity. Where the latter is saturated, some kind of parallel laying down of the "impurity" crystals may be possible. But what correlation of interionic distances can be possible in a given host crystal and such widely diverging molecules as are shown in Fig. 2? These are chosen because they all have an identical power in modifying the  $\{010\}$  surfaces of  $K_2CrO_4$  crystals. There is little in common between the molecules themselves, nor is the puzzle rendered any clearer when we place their modifying power towards  $\{010\}$  of  $K_2SO_4$  in juxtaposition. It is surprising that, if we allow, for a moment, cation-anion distances to have something to do with the problem, the results for the two cases are so different, for there can be only very minor differences between either configuration or dimensions in  $\{010\}$  of  $K_2SO_4$  and  $\{010\}$  of  $K_2CrO_4$ . Table II brings this into view.

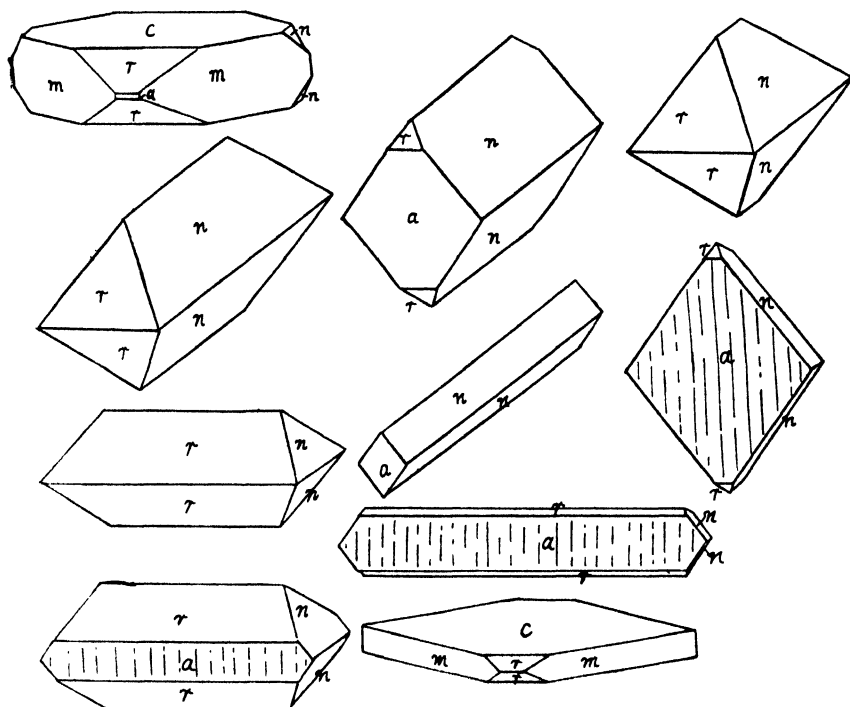


FIG. 3.—The various habit changes on potassium and ammonium perchlorate crystals.  
 $a = 100$ ,  $b = 010$ ,  $c = 001$ ,  $m = 110$ ,  $r = 102$ ,  $n = 011$ .

These same two planes, of practically identical configuration and size, provide further evidence against the view that cation-anion distances are a necessary feature of adhesion and habit change. For, as Table III shows (and it could be enlarged many times and for many crystals), these two similar faces can react, in the habit-modifying way, vastly differently with the same impurity. The figures in the last two columns indicate how many times more crystal material there is in solution than there is impurity, for a "standard" habit change; alternatively, 1 unit of the given impurity will give a "standard" of habit-modifying power (e.g., with  $010 = 011$ , a state readily detected by the eye) when so many units of crystal material are dissolved in the same solution. Evidently habit changes depend on

TABLE II  
(see also Fig. 2 for formulæ)

Colour Index, No.	Name	Effectiveness to {010} of $K_2CrO_4$	Effectiveness to {010} of $K_2SO_4$
34	Azo Orseille R (Action) .. .. .	All give 010 = 011 at about 1 in 5000 to 6000	1 in 250
47	Archil Substitute 3VN (St. Denis) ..		1 in 2000
438	Trypan Red (Meister, Lucius & Brüning)		1 in 40,000
670	Guinea Green 2G (Action) .. .. .		1 in 5000

TABLE III

Colour Index, No.	Name	{010} $K_2CrO_4$	{010} $K_2SO_4$
30	Fast Acid Magenta B (J. B. Sharp) .. .. .	20,000	500
31	Brilliant Lanafuchsine 2G (Casella) .. .. .	45,000	670
78	Scarlet G R (Action) .. .. .	22,500	800
180	Carmoisine L9156K (I.C.I.) .. .. .	25,000	670
184	Various, including Naphthol Red S (Badische) ..	25,000	5,000
637	Kiton Yellow S (Clayton Aniline) .. .. .	13,000	0
640	Various Tartrazines .. .. .	20,000	250
723	Chromazurol S. Conc (Geigy) .. .. .	7,500	0
737	Wool Green B S (Bayer) .. .. .	27,000	3,300
209	National Fast Wool Blue B (Allied Chem. & Dye Corp., U.S.A.) .. .. .	>0	15,000
401	Pontamine Diazo Black B H S W (du Pont) ..	500	25,000
438	Trypan Red (Meister, Lucius & Brüning) ..	6,000	40,000
735	Xylene Fast Green B (Sandoz) .. .. .	1,000	10,000

TABLE IV \*

(see Fig. 3)

EFFECT OF CONCENTRATION ON THE {011} AND {102} EFFECTS OF  $KClO_4$  AND  $NH_4ClO_4$ 

Colour Index, No.	Name	$KClO_4$		$NH_4ClO_4$	
		{011} effect	{102} effect	{011} effect	{102} effect
150	Orange I (St. Denis) .. .. .	200	40	2750	0
151	Various Orange II samples .. .. .	670	200	1450	600
161	Orange R (Clayton Aniline) .. .. .	1670	670	6700	5,000
184	Naphthol Red S (Badische) .. .. .	400	50	2500	150
284	Orseilline BB (Bayer) .. .. .	0	1,330	8000	0
313	Brilliant Croceine 9B (Casella) .. .. .	133	67	0	1,000
438	Trypan Red (Meister, Lucius & Brüning)	0	2,860	7000	0
456	Brilliant Congo R (Bayer) .. .. .	0	20,000	9000	7,000
511	Brilliant Azurine B (Bayer) .. .. .	0	3,000	0	45,000

\* The column figures have the same significance as in Table III except that two distinct "standard" habit changes, on {011} and {102}, are taken. These were explained in the writer's 1935 paper. They are the same "standards" for  $NH_4ClO_4$ , though the figures are usually not identical in the two cases.

something far trickier to specify than the simple conception we have just criticized, viz., cation-anion distances.

A further mystery of habit modification is the way in which certain substances can be modified in two different ways, not so much by two different impurities (later) as by two different concentrations of the *same* impurity, acting at the same temperature. The first example of this was pointed out by the writer in 1935.<sup>12</sup> Briefly, most dye impurities at low concentration cause the 001-110 habit of  $\text{KClO}_4$  crystals (Fig. 3) to assume an elongated shape on the  $x$ -axis due to the enlargement of the brachydome {011} (Fig. 3). Increase in concentration of dye, however, usually causes a new effect—on the macrodome {102}—to show itself and ultimately to prevail (Fig. 3). A few impurities possess the {011} influence only, while

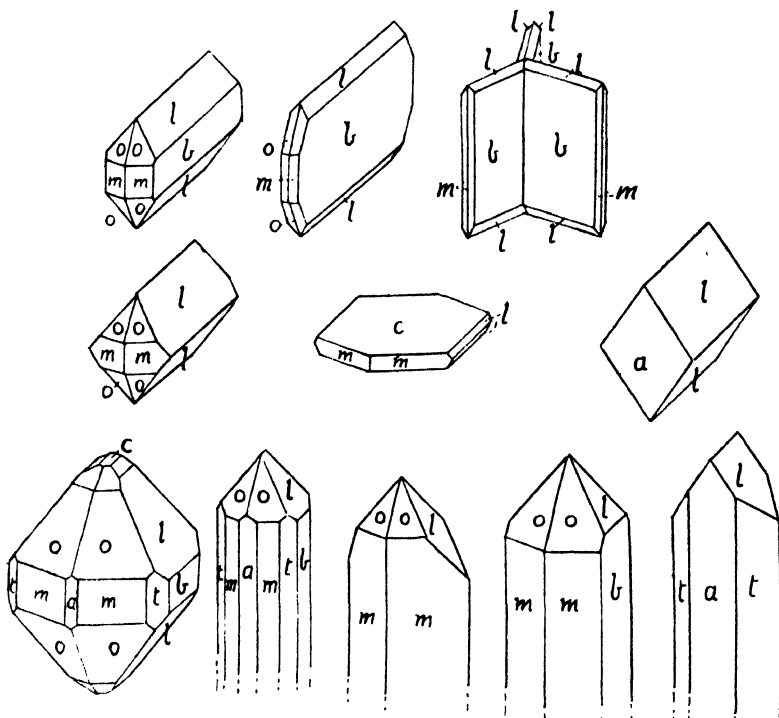


FIG. 4.—The various habit changes on potassium sulphate crystals.  
 $a = 100$ ,  $b = 010$ ,  $c = 001$ ,  $m = 110$ ,  $o = 111$ ,  $l = 021$ ,  $t = 130$ .

there are yet another few which influence {102} only. There are a few dyes with special effects, too, e.g., on {100} (large  $a$  in Fig. 3), but the majority of the several hundreds of dye impurities studied have the first-named effect at a lower concentration and the power to change over, when this is increased, to the other effect. It was, at the time, the writer's view that smaller dye impurities possessed the {011} effect, larger ones the {102} effect, and that increased concentration caused the lesser molecules to associate in solution and so to imitate the effect of the larger molecules. Results on  $\text{KClO}_4$  crystals pointed in an uncanny manner to this conclusion, even after over six hundred crystallizations had been performed. Yet it is inadmissible, as will be seen from a perusal of Table IV where a few results from  $\text{KClO}_4$  are compared with

<sup>12</sup> Buckley, *Z. Krist. A*, 1935, **91**, 375.

a few from the isomorphous crystals of  $\text{NH}_4\text{ClO}_4$ . It will be seen that not only are the respective effects, on  $\text{KClO}_4$  and  $\text{NH}_4\text{ClO}_4$  crystals, of the same dye in general not the same, but cases exist where a dye has nothing but the  $\{102\}$  effect on  $\text{KClO}_4$  and the  $\{011\}$  effect on  $\text{NH}_4\text{ClO}_4$  (e.g., Trypan Red, Orseilline BB). Whatever the cause of this change-over it cannot be explained in terms of associated molecules but must involve some complex relationship between the impurity and the two surfaces,  $\{011\}$  and  $\{102\}$ , for usually one is being influenced even when the other is being more effectively modified.

Another feature which requires discussion is one originated by France.<sup>10</sup> It is that, since the oncoming ions are being pulled in by the residual valency force fields of the various surfaces, those surfaces possessing the greater values of these force fields will be at a great advantage in the adsorption process and the ions will be preferentially adsorbed on them. Since, in general, those planes which are peopled by like ions will possess the biggest attractions to impurity ions of opposite sign, they will be the ones to suffer habit

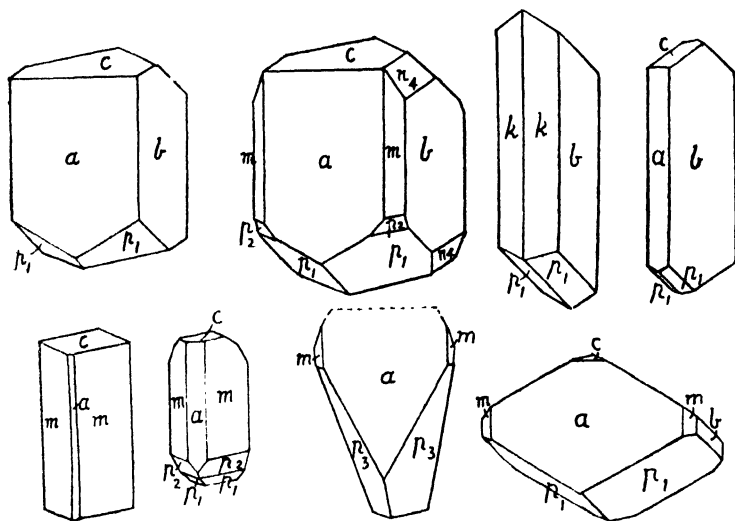


FIG. 5.—The various habit changes on borax crystals.

$a, b, c, m$ , the usual significance;  $p_1 = 11\text{T}$ ,  $p_2 = 22\text{T}$ ,  $p_3 = 33\text{T}$ ,  $n_4 = 041$ ,  $k = 320$ .

modification. France cites numerous examples from his own experience where such appears to be the case and, standing by itself, the idea seems plausible. Instances have been given, however, where crystals can adsorb on neutralized planes, e.g., Frondel's work<sup>13</sup> on sodium fluoride, etc. Dye was always adsorbed on the  $\{100\}$  surfaces although these are of the rocksalt pattern and even when habit changes are taking place on the adjacent  $\{111\}$  planes. France still considers the residual valency force fields to pull in the adsorbable ions but qualifies it by the remark that if the face, e.g., the unsaturated  $(111)$  surface here, after pulling the ions in cannot accommodate them to its configuration, they will seek to settle elsewhere, e.g., on the  $(100)$  (saturated) plane. It is probable that the adhesion of ions to crystal surfaces is not so simple as France has assumed, and his example of a non-adhering surface—the  $(100)$  of the  $\text{K}_2\text{SO}_4$  crystal—is not fortunate since the writer obtained both adhesion and habit modification on this face by means of the dye Alizarine

<sup>13</sup> Frondel, *Amer. Miner.*, 1940, 25, 91.

TABLE V  
SOME CRYSTALS WITH SEVERAL DIFFERENT HABIT CHANGES

Substance	Impurity and effect						
K <sub>2</sub> SO <sub>4</sub> ..	Most sulphionate dyes. On {010}	Acid Magenta (No. 692), Safranin (No. 841). On {110}-{010}	Alizarine Blue 3BR (No. 1088). On {110}	Alizarine Yellow 5G (No. 122). On {100}	Pigment Fast Yellow G (No. 651). On {100}-{130}	Bismarck Brown (No. 331). On {110}-{010} - {100}-{130}	S <sub>2</sub> O <sub>8</sub> <sup>2-</sup> ion S <sub>2</sub> O <sub>8</sub> <sup>2-</sup> ion. On {001}
KClO <sub>3</sub> ..	Most sulphionate dyes. On {011}	Chromotrope 2B (No. 45). No. 1053, 1054 On {101}	Jet Black R (No. 296), also dyes No. 450, 451, 452. On {001}	Cr <sub>2</sub> O <sub>7</sub> <sup>2-</sup> ion. On {010}			
NH <sub>4</sub> ClO <sub>4</sub>	Most sulphionate dyes. On {011}	Most sulphionate dyes. On {102}	Chromotrope 2B (No. 45), also No. 29, 91, 692, 1053, 1054, 1063. On {100}	Erio Fast Fuchsin BL (No. 758). Cr <sub>2</sub> O <sub>7</sub> <sup>2-</sup> ion. On {001} Striations    [010] or [110]	SO <sub>4</sub> <sup>2-</sup> ion, etc. (with KMnO <sub>4</sub> crystals only). On {110}		
Borax ..	Many sulphionate dyes. On {110}	Some sulphionate dyes (e.g., No. 172). On {110} + {221}	The Cu <sup>++</sup> ion On {010}	Vaccanine Blue (No. 135). {111}	Orange R (No. 161). On {100} and {331}; and slightly {110} (Rosettes)	Indian Yellow G (No. 146). On {320} + On {111}	
Potassium hydrogen tartrate	Many sulphionate dyes. {On 010}	Neutral Red, several basic dyes. On {010} + {0k1} elongated	Cu <sup>++</sup> ion on bisphenoid. {111}				
K <sub>2</sub> Cr <sub>2</sub> O <sub>7</sub>	Most sulphionate dyes. On {010}	Brill. Conc. 9B (No. 313), also No. 621, 622, 624, 138, 182, etc. On {111}	No. 195, etc. On {010} + {111} pseudo-prisms				





II *a*. "Flats" on  $\{010\}$  of  $K_2SO_4$ ; dye forms hour-glass on  $\{110\}$ .



II *b*. As II *a*; "hour-glasses" on  $\{111\}$ .

Yellow 5G (*Colour Index*, No. 122) in 1934.<sup>14</sup> Further, when a sufficiently large number of experiments are carried out with a large choice of materials, it will be found that it is the *rule*, rather than the exception, for a crystal to suffer modification in one of *several* ways.  $K_2SO_4$  and borax are good examples of this, though the latter is usually only influenced at great strengths of impurity (Fig. 4 and 5). Some of these variations are shown in Table V, and examples are known where habit change actually occurs on the predominant form of a crystal which is often presumed to be a neutral type of plane, e.g., on {010} of  $K_2Cr_2O_7$  and of potassium hydrogen tartrate, both of which are rendered even more extensive, and the crystals correspondingly thinner, by impurities.

We have cleared the ground by discussing various phenomena occurring during the growth of a crystal in the presence of impurity. We may then come to the most important point to be raised by the writer in this paper, viz., that no *direct* relationship can be traced between deposition of impurity on certain faces—whether in the manner advocated by the writer or by Bunn and Royer—and modification of habit. Hints of this possibility have already appeared in this paper, e.g., Walcott's observation<sup>6</sup> that barium nitrate crystals, modified by Methylene Blue to cube-habit, may yet be colourless, and the observation of Frondel<sup>10</sup> that whereas the habit change in NaF with Croceine Orange (*Colour Index*, No. 26) is from cube to octahedron, actual deposition is on {100}. But the point was first stressed in a paper<sup>15</sup> in 1934 by the writer, from observations on  $K_2SO_4$  crystals, that visible deposition could be quite unrelated to habit change. Photographs showed well-marked hour-glasses on {110} and on {111} of coloured dye which exhibited pleochroic changes, i.e., the dye ions had been incorporated in the host lattice in a regular manner, and yet there was an accompanying *strong* habit change, *not* on {110} or {111} but on {010}, and this had no adhesion of particles to it.

Plate II *a* shows  $K_2SO_4$  with Azo Orseille R (No. 34), tabular on {010} with strong hour-glass inclusions on {110}. Plate II *b* is  $K_2SO_4$  with Brilliant Congo R, also tabular on {010}.

Another observation supporting this view is that very frequently very small amounts of impurity are needed to cause strong habit changes, e.g.,  $KClO_3$  with Trypan Red (1 in 60,000 by weight of the latter or 1 molecule to 600,000—or more—of the salt). Again, Cotton Blue Conc. No. 1 (*Colour Index*, No. 707) with  $K_2CrO_4$  crystals at a concentration of 1 in 67,000, or Brilliant Azurine B (No. 511) with  $NH_4ClO_4$  at 1 in 45,000. At these concentrations the solution is nearly colourless and the crystals definitely so. It would appear then that, during growth, the following are possible, according to circumstances such as rate of growth, concentration of ingredients and relationship of structures: (i) formation of parallel growths (epitaxis); (ii) the deposition of ionic impurities in parallel positions in layers or hour-glasses, (iii) modification of the crystal habit. No necessary connection between the latter and either of the former two has yet been found though some kind of connection between (ii) and (iii) seems to be implied in the writer's many figures for the differing potencies of different molecular configurations, but whatever it is remains to be discovered.

If it should be admitted that there does not appear to be any alternative to adhesion of particles for explaining habit modification, we are still on the horns of a dilemma. For consider two adjacent faces. One of these has occasional impurity ions adhering to it which are ousted from time to time and the surface cleaned up. This ousting creates the delay which

<sup>14</sup> Buckley, *Z. Krist. A*, 1934, **88**, 122.

<sup>15</sup> Buckley, *Z. Krist. A*, 1934, **88**, 248.



results in a changed rate of growth. Round the corner or edge a far greater number of the same ions are adhering, but this time the surface does not adjust itself by clearing these out, but grows over them. Yet this growing over and including causes less change in the growth rate than the operation just described. Why doesn't the (010) face of  $K_2SO_4$  grow over the trivial number of ions which are reputed to cause the habit change (one to several hundred thousands of ions with Trypan Red, *Colour Index*, No. 438)? Again, with many faces of different crystals, habit change and inclusion occur together. Are there still two functions for the same ion or is the habit change in this case merely due to the delay caused by growing over and inclusion?

The two cases given in this paper show how, with Azo Orseille R (*Colour Index*, No. 34), habit change is on {010} and adhesion on {110}: with Brilliant Congo R (*Colour Index*, No. 456), habit change is on {010} and adhesion on {111}. Now in one case (possibly not unique, but the only one detected) habit change and inclusion were on the same {010} faces. This occurred with Columbia Green B, *Actiengesellschaft* sample of *Colour Index*, No. 593. This is readily decomposed by heat and the solution is difficult to prepare. It is an interesting problem.

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## THE EFFECT OF DYES ON THE CRYSTAL HABITS OF SOME OXY-SALTS

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The normal acicular crystals of ammonium nitrate are not well adapted to the uses of the salt in explosives, fertilizers, etc., and an extended investigation of the crystal habit modification of the salt by the presence of impurities in the saturated solution has been made. The impurities introduced included a comprehensive range of synthetic dyestuffs, and in all over 120 individual dyes were tested. In view of the very interesting effects obtained with the acid triphenylmethane dye, Acid Magenta, which are described below and in the following paper,<sup>1</sup> a similar search for crystal habit modificants of ammonium sulphate, potassium nitrate and sodium nitrate has been conducted.

The study of the crystal habit modification of ammonium nitrate is complicated by the range of polymorphs occurring between 169° C and room temperature. Lehmann<sup>2</sup> found that rosecobaltic nitrate modified the crystal habit of ammonium nitrate II, the tetragonal modification stable between 84.2° C and 125.2° C, while Bunn observed that the habit of the orthorhombic ammonium nitrate IV, stable between -18° C and 32° C and metastable up to 50° C, was modified from the normal {110} acicular prisms to platy crystals by crystallization in the presence of 0.10 % sodium

<sup>1</sup> Whetstone, This Discussion.

<sup>2</sup> Lehmann, *Z. Krist.*, 1887, **12**, 389.

hexametaphosphate.<sup>3</sup> Retgers<sup>4</sup> investigated the artificial colouring of ammonium nitrate, potassium nitrate and ammonium sulphate, among other substances, by dyes, but did not find any crystal habit modifications of these salts, apart from one isolated example, in the case of potassium nitrate. No previous account of the crystal habit modification of ammonium nitrate in the presence of dyestuffs has been found in the literature; the present paper deals with the crystal habit modification of ammonium nitrate IV.

Although France indicated that he intended to investigate the crystal habit modification of ammonium sulphate with dyes<sup>5</sup> no publication on this subject has been traced; this worker has, however, examined the isomorphous potassium sulphate<sup>6</sup> and has also investigated the crystal habit modification of a number of oxy-salts including sodium nitrate.<sup>7</sup> Buckley also investigated the crystal habit modification of potassium sulphate, and other oxy-salts, by dyes.<sup>8</sup> No record of any investigation of the crystal habit modification of potassium nitrate has been traced.

### Experimental and Results

The dyes used by Buckley and France in their investigations were mainly azo-dyes; Buckley in particular carried out intensive investigations, but only a few examples of dyes belonging to other classes are mentioned in his publications. In the present work, the dyes included a substantial number of triphenylmethane derivatives, while anthraquinone and other dye classes were also included in the selection examined. The dyestuffs used were mostly commercial samples, although a few were laboratory preparations. Their purity was thus in almost all cases unknown, and accordingly no attempt to obtain a quantitative relationship between the extent of the observed habit modifications and the dyestuff constitution has been possible.

In general, the concentration of dye used in the mother liquor in these tests has not exceeded 0.10 %. In many cases, however, the solubility of the dyes has been extremely low, owing to the high saline concentration in the salt solutions (this has particularly been the case with ammonium nitrate and ammonium sulphate) and the dyes have been used in saturated solution. No attempt in these cases has been made to estimate the dye concentration.

Tables I, II, III and IV summarize respectively the effects of dyes on the crystal habit of ammonium nitrate IV, ammonium sulphate, potassium and sodium nitrates, as encountered in these investigations. The full range of dyes tested is not included in these Tables: sufficient, however, of the dyes not found effective in crystal habit modifications has been included to give an indication of the complete range examined. In each Table, the first column indicates the dyestuff class, the second column the identification number of the dye in Rowe's *Colour Index*, the third the name of the dye, the fourth an approximate indication of the order of the solubility of the dye in the saturated salt solution, and the fifth gives a brief description of the types of habit modifications encountered. In contrast with the procedure of Buckley, who looked for a "standard" degree of habit modification given by a very small proportion of his most effective dyes, and who therefore obtained habit modifications of a similar order with many other dyes (especially because he was dealing with less soluble salts having a considerably smaller "salting-out" effect on the dyes than those considered here), we have aimed at recording the maximum habit modification obtainable, when the dye is present in saturated solution in the saline mother liquor, except for the most powerfully effective dyes, when 0.10 % solutions were used.

<sup>3</sup> Bunn (private communication).

<sup>4</sup> Retgers, *Z. physik. Chem.*, 1893, **12**, 614.

<sup>5</sup> France, *J. Alexander's Colloid Chemistry*, Vol. V (Reinhold, New York, 1944), p. 443.

<sup>6</sup> Righerink and France, *J. Physic. Chem.*, 1938, **42**, 1079.

<sup>7</sup> Weinland and France, *J. Physic. Chem.*, 1932, **36**, 2832.

<sup>8</sup> Buckley, *Z. Krist. A*, 1934, **88**, 122, 248, 381.

**TABLE I**  
**CRYSTAL HABIT MODIFICATION OF AMMONIUM NITRATE IV WITH DYES**

Dye class	Colour index number	Name of dye	Solubility*	Effect on habit of crystals
Nitroso-compounds	5	Naphthol Green BNS	S.	Tendency to {010} laths
Nitro-compounds	10	Naphthol Yellow S	V.S.S.	Tendency to {010} plates
Mono-azo	27	Naphthalene Fast Orange 2G	S.S.	Tendency to {010} laths
	79	Naphthalene Scarlet R125	V.S.S.	—
	89	Crystal Ponceau	S.S.	—
	153	Azofuchsine G	S.S.	Fibrous {010} crystals
	180	Carmoisine LS	V.S.S.	—
	182	Fast Red E	V.S.S.	—
	183	Croceine Scarlet 3BX	S.S.	—
	184	Edicol Amaranth	S.S.	Fibrous or platy {010} crystals
	185	Wool Scarlet 4R	S.S.	—
	186	Ponceau 6R	S.S.	—
Bis-azo	307	Coomassie Fast Black B	Insol.	—
	441	Chromazol Yellow CRS	S.S.	Tendency to produce {010} plates
	512	Chlorazol Blue R.W.	V.S.S.	—
	518	Chlorazol Sky Blue F.F.	V.S.S.	Some {010} tabular crystals
Pyrazolone	639	Lissamine Fast Yellow	S.S.	—
	640	Tartrazine	S.S.	—
Diamino-triphenyl-methane	666	Acid Green G C62961	S.S. S.	Platy {010} crystals
Triamino-triphenyl-methane	680	Methyl Violet (sulphonated)	S.	Tendency to {010} plates
	692	Acid Magenta	S.	Thin {010} plates
	694	Acid Violet 4RS	S.	Tendency to {010} plates
	706	Methyl Blue MBJ	S.S.	Tendency to {010} laths
Hydroxy-amino-triphenyl-methane	712	Disulphine Blue V	S.S.	Tendency to {010} laths
	715	Xylene Cyanol F.F.	S.S.	" " " "
Hydroxy-triphenyl-methane	—	Rosolic acid trisulphonate	S.	Tendency to {010} laths
	722	Solochrome Cyanine R.S.	S.S.	" " " "
Diphenyl-naphthyl-methane	734	New Patent Blue 4B	S.S.	—
	735	Lissamine Green V.S.	S.S.	Tendency to {010} laths
	737	Lissamine Green B.S.	S.S.	—
Xanthene	758	Fast Acid Violet R	V.S.S.	—
Oxazine	879	Gallophenine D	S.S.	—
Anthra-quinone	—	1 : 4-diamino-anthraquinone 2-sulphonate	S.	Tendency to {010} plates
	—	1 : 5-diamino-anthraquinone sulphonate	V.S.S.	—
	—	1 : 4 : 5 : 8-tetra-amino-anthraquinone sulphonated	S.S.	—

\* In all Tables, S. = soluble ; S.S. = slightly soluble ; V.S.S. = very slightly soluble.

TABLE II  
 CRYSTAL HABIT MODIFICATION OF AMMONIUM SULPHATE WITH DYES

Dye class	Colour index number	Name of dye	Solubility	Effect on habit of crystals
Nitroso	5	Naphthol Green BNS	S.	—
Mono-azo	27	Naphthalene Fast Orange 2G	S.	—
	89	Crystal Ponceau	V.S.S.	—
	153	Azofuchsine	S.S.	—
	182	Fast Red E	V.S.S.	—
	183	Croceine Scarlet 3BX	S.S.	—
	184	Edicol Amaranth	S.	Long fibrous flexible crystals
	186	Ponceau 6R	S.	—
Bis-azo	441	Chromazol Yellow CRS.	V.S.S.	—
	518	Chlorazol Sky Blue F.F.	S.	Dye adsorbed without modification
Pyrazolone	652	Eriochrome Red	S.S.	—
	639	Lissamine Fast Yellow	S.S.	—
	740	Tartrazine	S.	Long fibrous flexible crystals
Diamino-triphenyl-methane	666	Acid Green G	S.S.	—
	—	C62962	S.S.	—
	—	C62963	S.S.	—
Triamino-triphenyl-methane	657	Malachite Green (sulphonated)	V.S.S.	—
	680	Methyl Violet (sulphonated)	V.S.S.	—
	692	Acid Magenta	S.	—
Anthra-quinone		1:4-diamino-anthra-quinone 2-sodium sulphonate	V.S.S.	—
		1:5-diamino-anthra-quinone sulphonated	Insol.	—

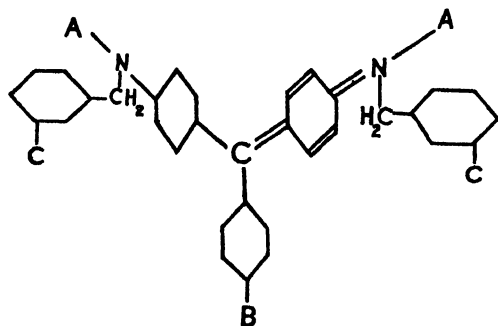
**Ammonium Nitrate IV.**—All the crystal habit modifications observed were due to the development of the {010} form instead of the usual {110} faces. This type of habit modification was very easily detected, since the crystals lying on a microscope slide gave acute bisectrix interference figures when examined conoscopically. The one common structural characteristic of the dyes found effective in bringing about the habit modification was the possession of sulphonate substituent groups, but all the dyes also contained at least one cationic substituent such as an hydroxyl or primary amino group.

It is of interest that Amaranth (184),\* representative of the mono-azo dyes containing a 3:6-disulphonated  $\beta$ -naphthol residue, and found effective by France in modifying the crystal habit of potassium sulphate and other salts, had a marked effect in modifying the habit of ammonium nitrate IV. The same steric factors as observed by France were found to deprive the  $\beta$ -naphthol residue of its habit-modifying powers, for the dyes Wool Scarlet 4R (185) containing a 6:8-disulphonated  $\beta$ -naphthol, and Ponceau 6R (186) containing a 3:6:8-trisulphonated  $\beta$ -naphthol residue, were quite ineffective. The most marked habit modification was obtained with the acid triphenylmethane dye, Acid Magenta\* (692), a mixture of trisulphonated rosaniline and *p*-rosaniline. Replacement of the amino substituent groups by hydroxyl groups, to give a trisulphonated

\* Numbers refer to the *Colour Index* (Rowe, 1924).

Rosolic Acid, reduced the extent of the habit modification, and a similar reduction was effected by methylation of the amino groups as shown by the dyes 693 and 694. Habit modification in a similar degree to that obtained in presence of the last two dyes was found to be produced by Methyl Violet (680) after sulphonation. Ethylation or benzylation of the amino group appeared to destroy the capacity of the dye for habit modification of ammonium nitrate IV. A few of the more complicated triphenylmethane dyes were found to have only a slight effect on the habit of the salt. Of a series of diamino-triphenylmethane acid dyes of similar character to Acid Green (666), only one (C62961) was found to have a marked effect on the habit of the salt, and further sulphonation of this dye appeared to negate this habit modification.

STRUCTURE OF EXPERIMENTAL DYESTUFFS C62959-C62963.



C62959 ; A =  $\text{C}_2\text{H}_5$ ;  
 C62961-C62963; A =  $\text{C}_2\text{H}_4\text{SO}_3\text{H}$ ;  
 C62959, C62961, and C62963 are sulphonated at B;  
 C62959, C62962, and C62963 are sulphonated at C.

A few apparently isolated dyes were found to possess fairly marked habit modifying properties, e.g., Chromazol Yellow CRS (441) a bis-azo dye, and 1:4-diamino-anthraquinone 2-sodium sulphonate; it is perhaps noteworthy that sulphonated 1:5-diamino- and 1:4:5:8-tetra-amino-anthraquinone did not modify the habit of the salt. Another example was the mono-azo dye Azofuchsine (153), and the bis-azo dye Chlorazol Sky Blue F.F. (518) gave a definite habit modification, the crystals of the salt in this case being coloured a deeper blue than the saturated saline solution of the dye.

**Ammonium Sulphate.**—The most marked habit modifications of this salt were obtained with the pyrazolone azo-dyestuff Tartrazine (640) and the mono-azo dyestuff Amaranth (184) containing the 3:6-disulphonated  $\beta$ -naphthol residue. As in the case of ammonium nitrate, the dyestuffs Wool Scarlet 4R (185) and Ponceau 6R (186) were not effective, again presumably because of the same steric factors. None of the other pyrazolone dyestuffs, in spite of their similarities to Tartrazine, was found effective in bringing about habit modification of ammonium sulphate. The habit modification appeared to be in the direction of the {001} form, identifiable by giving an obtuse bisectrix interference figure. Owing to the extreme thinness of the crystals obtained with 0.10 % of the above dyes, this deduction necessarily had to be made from observations of crystals from much more diluted solutions of the dyes, showing less extreme habit modification.

Definite indication of the formation of the {001} pinacoidal form was obtained in the presence of dyes such as Chlorazol Sky Blue F.F. (518) having a less marked effect on the habit of the crystals.

**Potassium Nitrate.**—In the case of this salt, habit modification was always found to be in the direction of producing plates of extended {001} form, which were easily recognized, since they produced an acute bisectrix interference figure on examination conoscopically. Amaranth was again effective in producing

TABLE III  
CRYSTAL HABIT MODIFICATION OF POTASSIUM NITRATE WITH DYES

Dye class	Colour index number	Name of dye	Solubility	Effect on habit of crystals
Nitroso	5	Naphthol Green BNS	S.	—
Mono-azo	27	Naphthalene Fast Orange	S.	—
	79	Naphthalene Scarlet 2R	V.S.S.	—
	153	Azofuchsine G	S.	—
	182	Fast Red E	S.	Strong tendency to {001} plates
	183	Croceine Scarlet 3BX	S.	—
	184	Edicol Amaranth	S.	{001} tabular crystals
	185	Wool Scarlet 4R	S.	—
	186	Ponceau 6R	S.	—
Bis-azo	441	Chromazol Yellow CRS	S.S.	—
	518	Chlorazol Sky Blue F.F.	S.	—
Pyrazolone	637	Hydrazine Yellow	S.	—
	639	Lissamine Fast Yellow	S.S.	—
	640	Tartrazine	S.	Tendency to {001} plates
Diamino-triphenyl-methane	666	Acid Green G	S.	—
	669	Acid Green M	S.	Tendency to {001} plates
Triamino-triphenyl-methane	692	Acid Magenta	S.	—
	707	Soluble Blue	S.	{001} tabular crystals
Hydroxy-triphenyl-methane	712	Disulphine Blue V	S.	—
	722	Solochrome Cyanine RS	S.S.	—
		Rosolic Acid Trisulphonate	S.	Thin {001} plates
Diphenyl-naphthyl-methane	735	Lissamine Green VS	S.	—
Anthra-quinone		1:4-diamino-anthraquinone 2-sodium sulphonate	S.	Thin {001} plates
		1:5-diamino-anthraquinone 2-sodium sulphonate	S.	—
		1:4:5:8-tetra-amino-anthraquinone sodium sulphonate	S.	Thin {001} plates

habit modification, and also several dyes of the Fast Red E type which contained a simple  $\beta$ -naphthol 6-sulphonic acid residue, while the sterically hindered azo dyes again had no effect. Tartrazine was effective; also, one or two triphenylmethane dyes were found capable of bringing about a somewhat similar habit modification; but the most advanced habit modification was obtained with two simple anthraquinone derivatives, 1:4-diamino- and 1:4:5:8-tetra-amino-anthraquinone sodium sulphonates, which gave highly coloured, thin plates.

**Sodium Nitrate.**—The typical habit modification of sodium nitrate as previously observed by France<sup>6</sup> lay in the appearance of {001} faces on the usual rhombohedra; the more marked the development of these faces, the greater was the tendency for the crystals to approach a platy habit. No outstandingly marked habit modification was observed. It is noteworthy that in

TABLE IV  
CRYSTAL HABIT MODIFICATION OF SODIUM NITRATE WITH DYES

Dye class	Colour index number	Name of dye	Solubility	Effect on habit of crystals
Mono-azo	27	Naphthalene Orange	S.S.	—
	53	Lissamine Violet	S.	—
	—	Aniline-sulphonate coupled with $\beta$ -naphthol 6-sulphonic acid	S.S.	Rhombohedral flattened {001} faces
	182	Fast Red E	V.S.S.	—
	184	Edicol Amaranth	Insol.	—
	185	Wool Scarlet 4R	V.S.S.	Slight tendency to produce {001} form
	186	Ponceau 6R	S.S.	
Bis-azo	346	Chlorazol Yellow	S.	—
	441	Chromazol Yellow CRS	S.	—
	518	Chlorazol Sky Blue F.F.	S.	{001} form developed on rhombohedra
Pyrazolone	636	Fast Light Yellow	S.	—
	640	Tartrazine	V.S.S.	—
Diamino-triphenyl-methane	666	Acid Green G	S.S.	—
	669	Acid Green	S.	—
	670	Acid Green GG extra	S.	Flattened rhombohedra {001} faces
		C62959	S.	Flattened rhombohedra {001} faces
		C62961	S.	—
Triamino-triphenyl-methane	692	Acid Magenta	S.	—
	707	Soluble Blue	S.	{001} form on rhombohedra
Hydroxy-triphenyl-methane	—	Rosolic Acid Trisulphonate	S.	—
	722	Solochrome Cyanine RS	Insol.	—
	715	Xylene Cyanol F.F.	S.	—
Diphenyl-naphthyl-methane	735	Lissamine Green V.S.	S.	—
Azine	861	Induline	S.	{001} form on rhombohedra
Oxazine	879	Gallophenine	S.	{001} form on rhombohedra

the change from the orthorhombic to trigonal system, the effectiveness of dyes such as Amaranth in modifying habit is apparently lost, and dyes such as Induline, Gallophenine, and Acid Green GG extra were found effective in bringing about habit modification.

### Discussion

The large variety of dyes found to be active crystal habit modificants, for each substance and also taken together, would appear to make it impossible to postulate a simple scheme to explain the effects of dyes in general in limiting crystal growth in certain well-defined directions in crystalline substances. Such a scheme must explain why, for instance, (a) Acid Magenta and Amaranth give marked habit modifications of ammonium nitrate IV, while Tartrazine has no effect, (b) Acid Magenta has

no effect on ammonium sulphate and potassium nitrate although Amaranth and Tartrazine both have marked effects on the habit of these salts, and (c) 1:4-diamino-anthraquinone 2-sodium sulphonate brings about marked habit modification of ammonium nitrate IV and potassium nitrate, yet does not affect ammonium sulphate. No theory involving the fitting of these dyes on one given crystal plane for each salt has so far been found capable of explaining these facts. It is noteworthy that in all cases examined, the crystal planes on which growth was limited by the dyes consisted of alternate positive and negative ions, which is contrary to the conception postulated from study of sulphates by France that planes consisting of like ions, i.e., of high potential, were preferentially affected by sulphonated dyestuffs. These observations are in agreement with the conclusions of Frondel<sup>9</sup> who studied the effect of dyes on a number of halides belonging to the cubic system.

We have frequently observed the inclusion of dye in growing crystals to give tinted zones, as noted by Buckley<sup>8</sup> and frequently the dye has been laid down on planes other than those most affected by the habit modification. In some cases zones of colour have been proved to be liquid inclusion, since regular etched cavities have been developed by heating the crystal on the microscope stage, e.g., hexagonal cavities were developed when potassium nitrate crystals modified by Tartrazine were so examined. However, in the case of ammonium nitrate IV modified with 1:4-diamino-anthraquinone 2-sulphonate and Acid Magenta, definite dye inclusions in the crystals have been noted.

It is considered that it is unlikely that a simple explanation of all the facts collected during work on crystal habit modification of salts in the presence of dyestuffs can at present be evolved. The mechanism of the effect of the dyes must be bound up with the actual mode of growth of the crystal planes, which itself can as yet be only inadequately explained. We are at present conducting an examination of the relationships between the ionic structure of crystal planes possibly concerned with dye absorption, and the constitution of dyes. It is possible to consider only the simpler dyes, but it is thought that the studies may at least prove of interest by showing clearer analogies between the structures of crystal planes and the dyestuff constitutions than have heretofore been suggested.

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<sup>9</sup> Frondel, *Amer. Miner.*, 1940, **25**, 91.

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## THE EFFECT OF CRYSTAL HABIT MODIFICATION ON THE SETTING OF INORGANIC OXY-SALTS

BY J. WHETSTONE

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It has been shown<sup>1</sup> that the caking of a water-soluble salt is a surface recrystallization phenomenon. The first stage in the caking process is the surface adsorption of moisture by the salt granules, or the migration to the

<sup>1</sup> Lowry and Hemmings, *J. Soc. Chem. Ind.*, 1920, **39T**, 101.



surface of moisture included in the granules during their preparation. Either process results in the production of a surface film of saturated solution which is concentrated by capillary forces at each point of contact between contiguous granules to form a liquid "bridge." Subsequent drying or temperature variation results in the partial or complete recrystallization of the dissolved salt into microcrystalline bridges which cement the granules together. The effect of this intergranular bonding occurring throughout a large package of ammonium nitrate, or other salt, is often to produce such hard caking that commercial handling difficulties are very much increased.

It is a common industrial practice suitably to modify the habit of certain crystalline substances, in the course of their manufacture, to improve their handling properties. Cases in point are the precipitation from solution in the presence of dextrin of lead azide to give rounded aggregates instead of the usual acicular crystals,<sup>2</sup> the crystallization of ammonium sulphate in the presence of magnesium sulphate and a trivalent metal sulphate to give crystals of controlled shape,<sup>3</sup> and the improvement in handling properties results from the modification in shape of these macroscopic crystals.

It has now been shown that if such a process of crystal habit modification is applied to a granular salt, it is sometimes possible to ensure that the solid bridges in the caked material are deposited in a fragile condition, so that the hardness of the bulk product remains low even after appreciable solution and recrystallization. This is a novel principle in the control of the caking of salts, and one of surprising efficacy.

### Experimental and Results

During the investigation of the crystal habit modification by dyestuffs and surface-active agents of ammonium nitrate and other oxy-salts, it was observed that coating ammonium nitrate crystals with 0.03 % of the dyestuff Acid Magenta brought about a most marked reduction in the setting tendency of the salt.<sup>4</sup> Acid Magenta has the effect of modifying the crystal habit of ammonium nitrate IV from the usual acicular {110} prisms to {010} plates, or minute "scales," and it was apparent that the dye coating of the macroscopic crystals provided a possible means of altering the crystal habit of the microcrystalline bonding between granules produced in the setting process. A number of other dyestuffs had also been found effective in modifying the habit of the salt to one with extended {010} form. These dyestuffs in general were not found to be capable of reducing the setting tendency of ammonium nitrate to nearly the same extent as did Acid Magenta.<sup>5</sup> It was therefore deemed of interest to investigate the relationship between the crystal habit of the microcrystalline bonding between set salt particles and the setting of the salt; and the investigations with ammonium nitrate were extended to include ammonium sulphate, potassium nitrate and sodium nitrate. An important preliminary was definitely to establish that the habit modification of ammonium nitrate IV with Acid Magenta was connected with the reduced tendency to set of the salt, surface-coated with the dye. The possibility that the freedom from caking was in some manner due to the presence of the sulphonated triphenylmethane residue and not to the habit modification was eliminated by the observation that an ammonium nitrate/potassium nitrate 90/10 solid solution (in which the ammonium nitrate was stabilized as the modification III)<sup>6</sup> set almost normally after the granules had been coated with 0.05 % Acid Magenta. Acid Magenta did not markedly affect the surface tension of saturated ammonium nitrate solution, thus it was unlikely that its effect lay in the reduction of the cross-sectional area of the liquid bridges between contiguous crystals of the moist salt. The dye was also extremely soluble in the salt solution, so that a mechanism depending on the deposition of solid Acid

<sup>2</sup> Miles, *Phil. Trans.*, 1935, **235**, 125.

<sup>3</sup> Roberts, *Gas World*, 1943, **119**, Coking Section 8.

<sup>4</sup> Whetstone and I.C.I., *Brit. Pat.*, Appl. No. 401/47.

<sup>5</sup> Whetstone and I.C.I., *Brit. Pat.*, Appl. No. 24829/47.

<sup>6</sup> Whetstone, *Can. J. Res. B*, 1948, **26**, 499.

Magenta particles on the surface of the salt granules was not acceptable. It was therefore concluded that the effect of Acid Magenta on the setting of ammonium nitrate must be a consequence of the habit modification brought about by the dye on the polymorph of the salt stable at normal temperatures.

Buckley, in his investigations of the effect of dyes on the crystal habit of potassium perchlorate, chlorate and sulphate<sup>7</sup> attempted to relate the degree of habit modification obtained with the proportion of dye in the crystallizing solution. In the control of setting, however, a saturated solution of the dye is almost certain to be present during the crystallization process, because even a dye concentration of 0.05 % on the surface of ammonium nitrate which later adsorbs 0.2 % of moisture could theoretically produce a solution containing nearly 10 % of dye, a proportion not approached even by Acid Magenta, at ordinary temperatures. Therefore, in the present work it was decided to study crystallization from solutions which were saturated with respect to the dyes, so that the results would be directly comparable with the recrystallization which occurs during the process of caking.

Caking tendency was estimated by preparing lightly compressed (10 lb./sq. in.) cylinders of the salt, 1½" diam., in porous paper wrappers, and allowing these to stand in a suitably humid atmosphere, the moisture uptake being controlled by time of exposure and observed by frequent weighing. Setting was induced, after the required moisture content had been reached, by drying the cylinders and the hardness was then determined with a simple penetrometer. Table I contains a summary of the relationships between moisture uptake and hardness of the set cartridges of the salts examined, in the presence of certain dyes.

**Ammonium Nitrate.** The relationship between the maximum effect of each dye on the crystal habit of ammonium nitrate IV and its effect on setting when coated in the proportion of 0.10 % on crystals of the salt was first investigated. No dye was found to be equal to Acid Magenta (which had a very high solubility (3-4 %) in the saturated salt solution and which produced the most extreme degree of crystal habit modification) in lessening the setting tendency of ammonium nitrate IV. A few other dyes, however, which in the crystallization experiments had been found capable of altering the habit of ammonium nitrate IV to thin plates, were found to give quite a marked reduction in the setting tendency of the salt. Probably the best of these was 1 : 4-diamino-anthraquinone 2-sodium sulphonate; but some triphenylmethane dyes similar to Acid Magenta save that the amino groups were alkylated, such as Red Violet 4 RS (694) or sulphonated Methyl Violet, were also quite effective. Dyes such as Amaranth (184) and the more complicated sulphato-triphenylmethane dyestuff "C62961" were rather less effective.

The dyes which brought about habit modification to prisms and laths, such as Chlorazol Sky Blue FF (518) and Chromazol Yellow CRS (441), had a smaller effect on the caking of ammonium nitrate. Results of nearly the same order were obtained with certain dyes which caused no habit modification and which merely exercised a surface-coating effect on the crystals.

It appeared, therefore, that the extent of the habit modification obtainable with saturated solutions of the dyes in ammonium nitrate solution could be linked with the improvement in setting obtainable when ammonium nitrate was coated with 0.1 % of the dye. Dyes capable of giving a very extreme habit modification were apparently most effective in controlling setting, whereas dyes giving slight habit modification had but little effect. The validity of this rather circumstantial conclusion has been tested by similar investigations to relate the maximum effect of dyes on crystal habit modification with the effect of dyes on setting when ammonium sulphate, potassium nitrate and sodium nitrate were coated with small proportions (0.10 % unless otherwise stated) of dyestuffs, applied from solution to the salt crystals.

**Ammonium Sulphate.** In the case of ammonium sulphate, the two dyestuffs Amaranth (184) and Tartrazine (640) which had been found to produce the most extreme habit modification (aggregates of thin flexible fibrous crystals in each case being deposited from solutions of ammonium sulphate containing 0.10 %

<sup>7</sup> Buckley, *Z. Krist.*, 1933, **85**, 58; 1935, **91**, 375.

TABLE I

EFFECT OF DYE TREATMENT ON SETTING OF SOME INORGANIC SALTS  
(Mesh figures given refer to B.S.S.)

Salt, Dye, Colour Index No., Quantity used	Moisture Contents and Setting Hardness					
	Mois- ture (0-0.5%)	Hard- ness	Mois- ture (0.5- 0.9 %)	Hard- ness	Mois- ture (above 1.0 %)	Hard- ness
AMMONIUM NITRATE * .. (30-60 mesh)	0.18	9	0.58	>18		
Acid Magenta (692) .. .. .	0.14	3	0.59	3	1.03	5
0.05 % .. .. .	0.21	2	0.73	5	1.49	6
1 : 4-diamino-anthraquinone 2-sulphonate 0.10 % ..			0.52 0.77	3 5	1.04 3.06	6 >18
Amaranth (184) .. .. .	0.19	5	0.54	8		
0.10 % .. .. .	0.37	5				
Chromazol Yellow CRS (441) 0.10 % .. .. .	0.28	10	0.55 0.60	13 >18		
Azofuchsine G (153) .. .. .	0.24	5	0.39 0.86	7 14	1.07	>18
AMMONIUM SULPHATE .. .. . (100 mesh)	0.250 0.458	13 >18				
Amaranth (184) .. .. .	0.29	0	0.89	0	1.79	6
0.10 % .. .. .	0.36	0			3.16	10
Azofuchsine G (153) .. .. .	0.310	5	0.50 0.89	13 >18		
0.10 % .. .. .						
AMMONIUM SULPHATE .. .. . (24 mesh)	0.34	8	0.58 0.81	12 >18	1.26	>18
Tartrazine (640) .. .. .	0.38	4	0.73 0.93	6 5	1.13 1.26	6 4
Chlorazol Sky Blue F.F. (518)	0.48	6	0.57	5	1.12	18
POTASSIUM NITRATE .. .. . (150-200 mesh)	0.199 0.316	>18 >18				
1 : 4-diamino-anthraquinone 2-sulphonate 0.10 % ..	0.37	3	0.66 0.53	5 5	1.67 1.97	5 8
Fast Red E .. .. .	0.21 0.35	6 6	0.86	7		
Tartrazine (640) .. .. .	0.104 0.35 0.457	5 6 6	0.64	>16		
0.10 % .. .. .						
Amaranth (184) .. .. .	0.20	8	0.71	>18		
0.10 % .. .. .	0.44	9	0.92	>18		

\* Plant product with 1 % china clay.

of these dyes) were also found to give a most marked reduction of the setting tendency of the salt, which effect was not equalled by any other dyes investigated.<sup>a</sup> Such dyes as Chlorazol Sky Blue F.F. (518) and Azofuchsine G (153), which had been found to give a less marked effect on the crystal habit of the salt, had but a slight effect on its setting tendency.

**Potassium Nitrate.** The two dyestuffs, 1:4-diamino-anthraquinone 2-sodium sulphonate and 1:4:5:8-tetra-amino-anthraquinone sodium sulphonate, which had been found to have the most extreme effect on the habit modification of potassium nitrate, giving thin plates tending to become hexagonal in outline, were found to be more effective in reducing the setting tendency of the salt than any other of the dyes examined; <sup>b</sup> while dyes such as Tartrazine (640) and Amaranth (184) which gave a less advanced crystal habit modification of the salt than the above anthraquinone derivatives did not reduce the setting tendency of potassium nitrate to the same extent. The disparity between the effects of the best dyes in reducing setting and those found less effective was probably not as marked as in the cases of ammonium sulphate and ammonium nitrate.

**Sodium Nitrate.** No dyestuff has yet been found capable of giving a marked reduction in the setting tendency of sodium nitrate. It is a noteworthy fact, therefore, that no dye has been observed to modify the crystal habit of the salt in an extreme degree.

### Discussion

It is considered probable that the effect of a crystal habit modificant in reducing the setting of salts such as those examined is connected with its tendency to produce modified crystals of extreme thinness in the micro-crystalline bonding between granules of the set salt. It has so far proved impossible directly to examine the intergranular bonds and therefore to verify this deduction. However, consideration of the nature of the habit-modified crystals obtained from experiments involving the crystallization from solution of the salts in presence of dyes provides strong presumptive evidence that the effect of the dyes found most effective in reducing setting, such as Acid Magenta for ammonium nitrate, Amaranth for ammonium sulphate, and 1:4-diamino-anthraquinone 2-sodium sulphonate for potassium nitrate, is due to the extreme thinness and fragility of the platy crystals produced in their presence. The crystals obtained from ammonium nitrate solutions at the ordinary temperature in the presence of Acid Magenta in proportion about 0.10 %, for instance, when wet are extremely soft, and when dry are quite friable. None of the other crystal habit modificants examined with ammonium nitrate produced crystals of comparable fragility and it is extremely probable that the effect of Acid Magenta on the setting of ammonium nitrate is simply to reduce the mechanical strength of the intergranular bonding by modification of its microcrystalline structure. The other crystal habit modificants examined reduce the mechanical strength of the bonding in less degree according to the thinness of the crystals produced in their presence.

Similarly, in the case of ammonium sulphate, the crystals obtainable when Amaranth or Tartrazine is dissolved in the crystallizing solution are of extreme flexibility when wet and are extremely fragile when dry, and the action of these dyes in combating setting is presumably similar to that deduced for Acid Magenta with ammonium nitrate. The thinness of the crystals produced by potassium nitrate in the presence of 1:4-diamino-anthraquinone 2-sodium sulphonate and 1:4:5:8-tetra-amino-anthraquinone sodium sulphonate is perhaps not quite so marked; but these dyes have a much greater habit-modifying effect for this salt than any others examined and were found to produce the greatest reduction in setting tendency.

<sup>a</sup> Butchart, Whetstone and I.C.I., *Brit. Pat.*, Appl. No. 31726/48.

<sup>b</sup> Butchart and I.C.I., *Brit. Pat.*, Appl. No. 33380/48.

A crystallographic examination of the crystals of extremely modified habit is instructive in explaining their fragility. The ammonium nitrate crystals all showed an extended  $\{010\}$  pinacoidal form, the potassium nitrate crystals a  $\{001\}$  form, whilst the ammonium sulphate crystals are regarded as having an extended  $\{001\}$  form since this occurred in partially modified crystals grown from solutions containing only one five-hundredth of the quantity of dye required for saturation. Cleavage planes of these salts are given as distinct (010), perfect (011), and distinct (001), respectively, by Winchell.<sup>10</sup> Ammonium nitrate IV shows relatively good (010) cleavage, as would be expected from consideration of its structure. Ammonium sulphate crystals show a fairly good and distinct (001) cleavage; consideration of the structure of this salt would, however, lead one to expect a ready (100) cleavage, and in fact this was found to be the case, the crystals easily cleaving parallel with (100) to give sections showing a central "acute bisectrix" interference figure when examined conoscopically. Ammonium sulphate also cleaved, less readily, on (010). The cleavage planes in potassium nitrate were less apparent than in ammonium sulphate, the (011) cleavage given as "perfect" in Winchell's book was not easily recognized, but fairly distinct cleavages parallel with (010) and (110) were observed. There appeared to be no cleavage parallel to (001).

Thus, in the cases of both ammonium sulphate and ammonium nitrate IV, the facial development of the crystals of modified habit is parallel with the principal cleavage plane. The fracture of the modified ammonium nitrate IV crystals therefore can only take place by separating planes of ions having relatively strong mutual attractions, since there is apparently no marked cleavage plane intersecting the faces of the habit modified crystals. Hence arises the importance of the extreme thinness of the crystals modified by Acid Magenta in conferring sufficient fragility to allow the dye to affect the setting tendency of this salt.

In the case of ammonium sulphate, crystals modified by Amaranth or Tartrazine show face development parallel with the main cleavage plane (001), but in addition the (100) cleavage is perpendicular to the direction of elongation of these narrow blade-like crystals. This confers the property of extreme flexibility on these crystals and increases their friability when dry. Since the (100) cleavage is less marked than the (001), however, dyes not producing crystals which are extremely thin perpendicular to the (001) plane do not tend to give as marked a degree of resistance to setting of ammonium sulphate as do Tartrazine and Amaranth.

The habit modified crystals of potassium nitrate are developed facially in a plane intersected by all cleavage planes of the salt. Thus the fragility of the platy crystals obtainable with 1 : 4-diamino-anthraquinone 2-sulphonate and one or two other dyes is sufficient to allow a marked effect to be exerted by these dyes on the setting of the salt, in spite of the fact that these habit modified crystals are not as thin as those obtainable with Acid Magenta and ammonium nitrate IV, or with Amaranth and ammonium sulphate.

This process of setting-control by the treatment with crystal habit modifiers of crystalline substances is clearly capable of extension to substances other than those examined, provided that suitable dyes or other modifiers can be found.

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<sup>10</sup> Winchell, *Microscopic Characters of Artificial Minerals* (T. Wiley and Sons, New York, 1931).

# GROWTH AND DISSOLUTION OF CRYSTALS UNDER LINEAR PRESSURE

BY CARL W. CORRENS

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Two problems are closely connected with crystal growth concerning which much obscurity exists still. It is strange that the connection between the two problems has only recently been established although both are the expression of the same laws. The two problems are, on the one hand, the pressure exercised by growing crystals and, on the other, the dissolution of crystals under the influence of linear pressure. In this connection by growth pressure (*Wachstumsdruck*) is meant only the property of a growing crystal to lift an imposed weight; volume changes occurring during phase changes (ice→water), with crystallization in saturated solutions, or with hydration are excluded here. Large effects have been attributed to this growth pressure especially in connection with the formation of ore-veins. The dissolution under linear pressure (the so-called Riecke's principle) has been made use of by Becke<sup>1</sup> to explain the tabular and platy habit of minerals in crystalline schists.

The Riecke principle,<sup>2</sup> a principle already formulated by Thomson,<sup>3</sup> states that under linear pressure a crystal has a lower melting point or a greater solubility respectively than an unpressed crystal. In aqueous solutions (which are considered here) a crystal under pressure is therefore in equilibrium with a solution which is supersaturated for crystals not subject to pressure. The equation describing this relation has been fully worked out in<sup>4</sup> and reads as follows:

$$RT \ln c/c_s = vP,$$

where  $c$  is the actual concentration and  $c_s$  the concentration when saturated,  $v$  the mole volume of the crystal substance and  $P$  the pressure.

This equation can be developed in a more elegant way, which will be outlined here. I am indebted to Prof. R. Becker for this contribution. The condition for equilibrium can be obtained by calculating in two different ways the amounts of work which are necessary to transform isothermally and reversibly a supersaturated solution (with osmotic pressure  $p$ ) into a saturated solution (with osmotic pressure  $p_s$ ). According to the principle of maximum work both are taken to be equal.

(1): The crystal grows under the load  $B$  and is enlarged by a layer of thickness  $h$ , which consists of  $n$  mole. The work gained is

$$A_1 = Bh$$

(2): A given quantity of supersaturated solution, which contains exactly  $n$  mole, is diluted until it has the osmotic pressure  $p_s$ . The dilution must be performed reversibly, for instance, by use of a piston which is only permeable to the solution. The work gained is

$$A_2 = nRT \ln p/p_s.$$

<sup>1</sup> Becke, *Denkschr. Akad. Wiss. Wien*, 1903.

<sup>2</sup> Riecke, *Nach. Ges. Wiss., Göttingen, Math.-Phys. Klasse*, 1894, p. 278.

<sup>3</sup> Thomson, *Phil. Mag.*, 1862, **24**, 395.

<sup>4</sup> Correns and Steinborn, *Z. Krist. A*, 1939, **101**, 117.

According to the principle of maximum work,  $A_1 = A_2$ .  
Therefore

$$Bh = nRT \ln p/p_s.$$

If  $P$  is the pressure on the crystal and  $q$  the surface area under pressure, then  $B = Pq$ ;  $qh$  is the volume of the deposited crystalline substance; by dividing by the number of moles  $n$ , the mole volume of the crystalline substance,  $v_{\text{solid}}$ , is obtained. The above equation therefore takes the form:

$$Pv_{\text{solid}} = RT \ln p/p_s.$$

If instead of the osmotic pressures the concentrations  $c$  and  $c_s$  respectively are taken, the same equation as given on p. 267 is derived:

$$Pv_{\text{solid}} = RT \ln c/c_s.$$

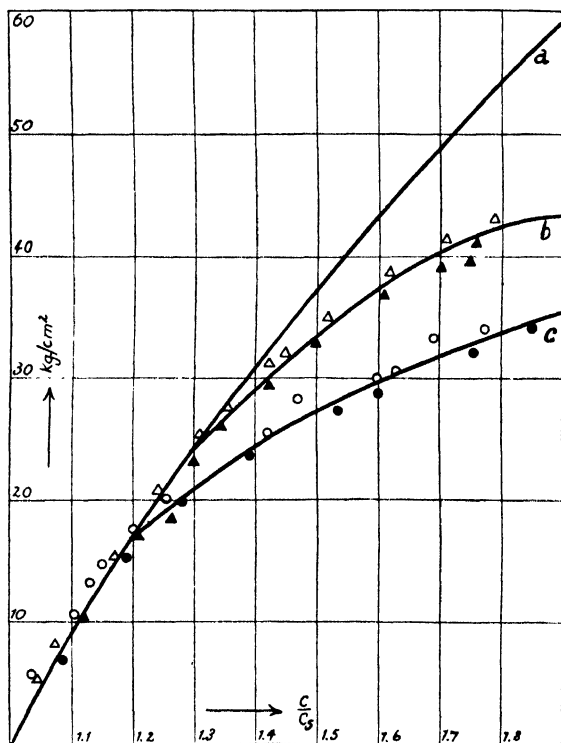


FIG. 1.—Relationship between pressure of growth and supersaturation (alum, 20° C solution stirred).

- (a) calculated curve,
- (b) observed curve for {111} ( $\Delta$  no growth,  $\blacktriangle$  growth),
- (c) observed curve for {110} ( $\circ$  no growth,  $\bullet$  growth).

The pressure which a growing crystal can exercise, e.g., by lifting a weight against gravity, depends on the degree of supersaturation. In addition to this, no unpressed crystals must be present in the solution, because they would remove the supersaturation, i.e., they would grow while the pressed crystals would dissolve. This brings us to the Riecke principle.

Experiments to determine the pressure of growth at various supersaturations were initiated in 1938 in collaboration with Steinborn<sup>4</sup> and have been carried out by means of a very sensitive pressure-balance (*Druckwaage*);

all precautions have been taken, such as, for example, maintaining constancy of temperature, checking of the degree of supersaturation, etc. The balance was constructed in such a way that by means of a mirror device a scale reading of 15 mm. corresponded to  $1 \mu$  linear growth. Pressures at which with a given supersaturation growth and no growth could be observed have been determined. Fig. 1 gives the results for various crystal planes of alum when the corresponding planes are between glass plates, and also gives an order of the magnitude of the maximum pressures which a growing crystal of alum can exert. Curve (b) which corresponds to the octahedron face  $\{111\}$  coincides at first with Curve (a) which has been calculated from the above equation, but for high pressures it departs from the calculated curve. Curve (c) for the rhombic dodecahedron faces  $\{110\}$  shows departures at lower pressures. The cube faces do not show any growth. From this it is seen that in addition to supersaturation and the absence of unpressed crystals there must be another condition in order that the crystal shall be able to lift a weight. This condition was pointed out in 1926<sup>5</sup> when I showed that loaded octahedrons of alum situated between mica plates cannot grow in the direction of the pressure.

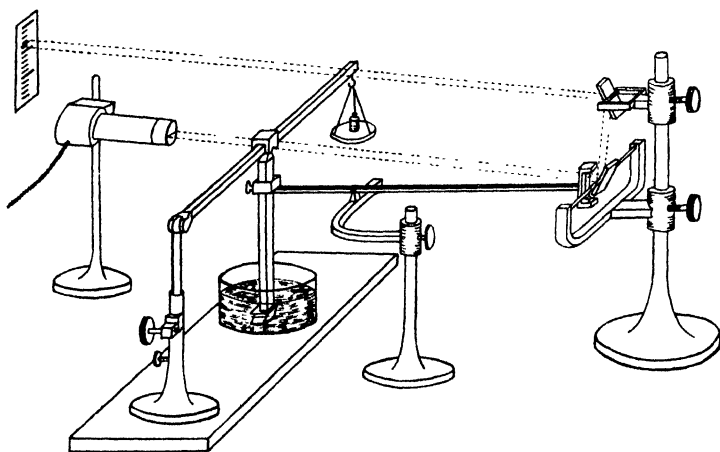


FIG. 2.—Sketch of apparatus measuring dissolution under the influence of linear pressure.

If the phase-boundary force between crystal (a) and solution (b) is  $\sigma_{ab}$ , that between the substance of the plates (c) (under and above the crystal) and the solution (b) is  $\sigma_{bc}$  and that between crystal (a) and substance (c) is  $\sigma_{ac}$ , then the following relation must hold true in order that the crystal might grow :

$$\sigma_{ac} > \sigma_{bc} + \sigma_{ab}.$$

It is only when  $\sigma_{ac}$  is greater than the sum of the two other phase-boundary forces that it is possible for solution to enter between crystal and the top and bottom plates, i.e., the crystal will grow. Unfortunately no quantitative data are available for these phase-boundary forces and their dependence on pressure. Attempts to obtain such data by means of growth experiments such as described here were unsuccessful. But from quite elementary ideas it is obvious that a crystal face can only grow if it does not grow tightly onto the underlying plate, which is the case with cubes of alum between

<sup>5</sup> Correns, *Sitz. Ber. Preuss. Akad. Wiss.*, 1926, **11**, 81.



glass plates. As Fig. 1 shows, phase-boundary forces or the ability to grow tightly onto the plate are different for the various faces of alum; they are also different for different plate materials which, of course, is shown by the fact that between gypsum plates both cubes and rhombic dodecahedrons do not grow.

These experiments have also a bearing on the problem of inclusions in crystals. Whether a neighbouring crystal is included in the growing crystal or pushed aside depends on the phase-boundary forces or, in other words, on the structure of the planes which are in contact. In this latter case the supersaturation must be great enough so that crystal growth is not stopped by the counteracting pressure (self-cleaning process of crystals).

During the last few years these experiments have been continued in collaboration with Brehler. The aim was to obtain data concerning the dissolution of crystals under linear pressure in saturated and supersaturated solutions. Some experimental difficulties were encountered; e.g., bending

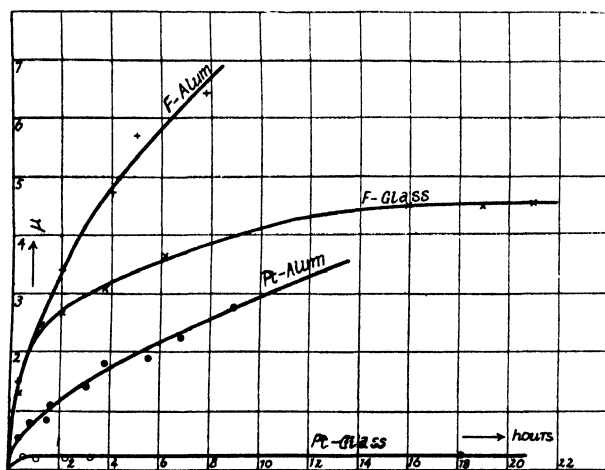


FIG. 3.—Dissolution of alum {111} between filter-paper and platinum sheets respectively compared with a glass cube instead of alum (load ca. 10 kg./cm.<sup>2</sup>, 15° C, slightly supersaturated and stirred solution).

in parts of the loading and measuring device may cause a diminution in height and thus give the illusion of dissolution. This did not matter in the growth experiments but must be eliminated in experiments by which dissolution was determined. Therefore a special apparatus shown in Fig. 2 in which this source of error was practically eliminated (1  $\mu$  growth or dissolution corresponds to 23 mm. on the scale) has been constructed. In experiments on dissolution it has to be borne in mind that, from the original crystal faces, different faces may be formed during dissolution. During growth, the faces (111), (110) and (100) of alum continue to grow during the duration of the experiment; it is with dissolution that the quick formation of new and different parts of crystal faces starting especially from etch grooves has to be counted upon. These faces have a structure and a phase-boundary force different from the original faces. Thus, octahedrons of alum placed between glass plates scarcely show any dissolution at all under linear pressure in slightly supersaturated solution (while the faces on the sides go on growing). This means that the new faces which have been formed at the commencement of dissolution grow tightly onto the glass. If, however, a layer of filter-paper is put between the glass and the octahedron

of alum or, if instead of glass, platinum sheets are used, dissolution can be demonstrated as is shown by Fig. 3. (Here the lowest curve, obtained with a glass cube and filter-paper, illustrates the bending of the apparatus and the compressibility of the filter-paper.)

From these experiments it is seen that the phase-boundary forces, i.e., the structure of the formed crystal face, play an even greater role in dissolution than in growth. This is because on dissolution many different sorts of crystal faces (generally of higher indices), of which one or the other can grow onto an under- or overlying plate, are found. It is also important to note that in those experiments in which dissolution under pressure has been observed, using filter-paper or platinum sheets, an increase in weight of the pressed crystal has been found, which proves that it has grown sideways. This observation could be taken to explain "schistosity" developed in crystalline schists under the influence of linear pressure; if not, doubts would arise as to whether, in silicate- and carbonate-minerals with their oxygen lattice planes, any crystal planes can exist which allow penetration of solution and consequently dissolution.

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## ON THE DISORDERING OF SOLIDS BY ACTION OF FAST MASSIVE PARTICLES<sup>1</sup>

BY FREDERICK SEITZ

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**Introduction.** Many solid materials undergo extensive changes when exposed to radiations of the various types that are available at the present time.<sup>2</sup> Undoubtedly the most striking and useful effect of this kind that has been studied to date is that observed in the silver halides when they are exposed either to electromagnetic radiations or to the various charged radiations that may be produced. The widespread availability of high-intensity sources of radiations of massive particles as a result of the development of electronuclear machines and neutron reactors now makes it possible to extend the field of study to regions that were hitherto closed. It is with this subject that we shall deal here briefly.

The beautiful theoretical work of Mott and Gurney<sup>3</sup> has shown that the decomposition which occurs in the silver halides when they are exposed to light or other charged radiations can be explained on the basis of a two-stage reaction. The incident radiation produces free electrons which wander about

<sup>1</sup> This paper is based on the declassified notes of a lecture series presented at Oak Ridge National Laboratory during the winter of 1946-47. It may be regarded as an extension of the work described by Prof. M. Burton in *J. Physic. Chem.*, 1947, **51**, 611. The writer is indebted to many of the colleagues listed by Prof. Burton for extensive discussions of the field during recent years. Most particularly he is indebted to Prof. E. P. Wigner who first called attention to this important aspect of radiation studies and made the initial quantitative estimates.

<sup>2</sup> Burton and Rollefson, *Photochemistry* (Prentice Hall, New York, 1939). Lind, *Chemical Effects of Alpha Particles and Electrons* (Chemical Catalogue Company, 1928).

<sup>3</sup> Mott and Gurney, *Electronic Processes in Ionic Crystals* (Oxford Univ. Press, 1940).

the crystal and become trapped; the trapped electrons attract mobile silver ions toward them and hence cause a separation of silver and chlorine atoms from one another. At least in the case in which the decomposition is caused by light quanta or  $\beta$ -rays, the constituent particles do not possess sufficient momentum to cause a relative displacement of atoms; this displacement occurs only because the ions are made mobile by thermal fluctuations. The separation is greatly retarded if the salt is cooled to liquid-air temperatures. On the other hand, fast massive particles such as  $\alpha$ -rays, protons, neutrons and fission fragments possess sufficient momentum that they may cause displacements directly and hence induce effects at any temperatures. Hence radiations of this type may be expected to induce atomic rearrangements in materials which are normally very stable and would not be influenced readily by the particles possessing a low momentum.

It is evident that the properties of any solid will be drastically altered if an appreciable fraction, say, 10 %, of its atoms are displaced and if the back-diffusion is not sufficiently rapid to undo the influence of the displacements. Thus a systematic study of materials in which atoms have been displaced, with particular emphasis on the change of properties and the manner in which these changes are altered as a result of back-diffusion, offers a promising means of obtaining further information concerning the properties of solid phases.

We shall consider the magnitude of the number of displaced atoms that can be induced in typical cases in the present paper. In connection with this problem, it is convenient to characterize the state of motion of any massive particle by giving the energy that an electron would have if it possessed the same velocity. This parameter, which will be designated by  $\epsilon$ , evidently is

$$\epsilon = \frac{m}{M} E, \quad . \quad . \quad . \quad . \quad . \quad (1)$$

in which  $E$  is the kinetic energy of the particle,  $m$  is the electron mass and  $M$  is the mass of the heavy particle. Generally speaking we shall be interested in massive particles which have velocities of the order of  $10^9$  cm. per sec. or less so that they are in the non-relativistic range. The parameter  $\epsilon$  will be about 10,000 eV or less. For example, the value of  $\epsilon$  associated with a 4 MeV  $\alpha$ -particle is about 545 eV, whereas that associated with a 40 MeV proton is 21,800 eV.

**Influence of Charged Particles.** A moving charged particle which is passing through matter dissipates its kinetic energy in two ways: by transmitting kinetic energy to the nuclei of the stationary atoms in elastic encounters in which the moving particle enters the strong coulomb field near the nucleus of the atom; and by exciting and ionizing the electrons which are attached to the atoms. We shall regard the moving particle as a complex atom to which a number of electrons are attached since this is the situation in which we shall normally be interested. In actual fact, the case in which the moving atom has such a low velocity that it is practically neutral is of very great interest. In this event the internal electrostatic field of the moving atom rather than the long-range coulomb field is most effective in inducing the transitions through which it loses energy.

(i) **Elastic Collisions.** The kinetic energy  $\Delta E$  which the initially stationary nucleus receives from the moving particle as the result of the elastic encounter is

$$\Delta E = E \frac{4 \mu^2}{M_1 M_2} \sin^2 \theta/2 \quad . \quad . \quad . \quad . \quad (2)$$

Here  $E$  is the kinetic energy of the moving particle,  $M_1$  and  $M_2$  are respectively the masses of the moving and stationary atoms,  $\mu$  is the reduced mass and  $\theta$  is the angle through which the moving particle is deflected in a co-ordinate system in which the centre of gravity is at rest.

The cross-section for the collision may be expected to correspond to Rutherford scattering for large angles of deflection since the unscreened nuclear charges will face one another at the point of closest approach in such collisions. The problem of obtaining a complete expression for the cross section can be treated in a satisfactory semi-quantitative fashion with the use of the perturbation methods derived from quantum mechanics by Born, provided the interaction potential between the moving particle and the atom is known. If  $V(r)$  is the interaction potential, the cross-section per unit solid angle for an elastic encounter in which the moving particle is deflected through angle  $\theta$  in the centre of gravity system is <sup>4</sup>

$$I(\theta) = \left| \frac{8\pi^2\mu}{h^2} \int_0^\infty \frac{\sin Kr}{Kr} V(r)r^2 dr \right|^2 \quad (3)$$

Here  $\mu$  is the reduced mass of the two particles,  $h$  is Planck's constant,  $r$  is the distance between the moving and fixed atoms and

$$K = \frac{4\pi\mu}{h} v \sin \theta/2,$$

in which  $v$  is the velocity of the moving particle.

It is very convenient for general purposes to express  $V(r)$  in a form which can be used for arbitrary pairs of atoms, gaining in generality what is lost in precision. The atom model of Fermi and Thomas provides a very useful basis for the determination of such a potential. In fact a good representation for the case in which the moving atom is nearly neutral is found to be

$$V(r) = \frac{Z_1 Z_2 e^2}{r} \chi(y Z_1^{1/2}) \cdot \chi(y Z_2^{1/2}) \quad (4)$$

in which  $Z_1$  and  $Z_2$  are the atomic number of the moving and stationary atoms,  $\chi$  is the Fermi-Thomas function for a neutral atom and

$$y = r/\beta, \quad \beta = 0.8853 a_0 \quad (5)$$

where  $a_0$  is the radius of the first Bohr orbit in hydrogen. The potential (4) approaches the pure coulomb potential when the moving and stationary atoms are very close and approaches zero as a result of electronic screening when the atoms are far apart. The form given is particularly appropriate when the value of  $\chi$  for the moving atom is of the same order of magnitude or less than the ionization energy of its valence electrons. The moving atom will then behave as if it were electrostatically neutral.

If (4) is inserted in (3) and the resulting cross-section is used to compute the rate at which the moving atom loses energy in travelling unit distance through a monatomic medium, it is found that to a good approximation this rate of loss is

$$-\left(\frac{dE}{dx}\right)_c = \frac{2\pi Z_1^2 Z_2^2 e^4 N_0}{M_2 v^2} \log E/E^* \quad (6)$$

Here  $N_0$  is the density of stationary atoms,  $M_2$  is their mass and

$$E^* = 0.618 (Z_1^{1/2} + Z_2^{1/2})^2 \frac{m M_1}{4\mu^2} E_R \quad (7)$$

in which  $M_1$  is the mass of the moving particle,  $m$  is the electronic mass and

<sup>4</sup> Mott and Massey, *Theory of Atomic Collisions* (Oxford Univ. Press, 1934).

$E_R$  is the Rydberg energy (13.54 eV).  $E^*$  is of the order of 0.01 eV for typical cases.

The energy dissipated in this way will produce two effects. First, it will generate lattice waves which eventually degenerate into thermal motion. Second, it will eject atoms from their normal lattice position, thereby forming vacancies and interstitial atoms. It is clear that the second of these two processes can occur only if the stationary atom receives a certain minimum energy  $E_d$  which is of the same order of magnitude but larger than the energy that would be required to remove the atom adiabatically from a normal lattice site to an interstitial position. We may expect  $E_d$  to be of the order of 25 eV for any atom which is bound to its position with an energy of the order of 10 eV, as is true of the most firmly bound metals, salts and valence crystals. For simplicity, we may assume that any atom receiving an energy larger than  $E_d$  actually is permanently displaced to an interstitial position. In this case, the rate at which energy is dissipated in producing such *displacements* is

$$-\left(\frac{dE}{dx}\right)_{c,d} = \frac{2\pi Z_1^2 Z_2^2 e^4 N_0}{M_2 v^2} \log \left( \frac{E}{E_d} \cdot \frac{4\mu^2}{M_1 M_2} \right) \quad (8)$$

The remaining part of (6) is expended in producing vibrational waves. It is interesting to note that the encounters between moving and stationary atoms in which the energy transferred is larger than  $E_d$  are invariably so close that the nucleus of each atom is inside the screening electron cloud of the other at the distance of closest approach. The average kinetic energy transferred to the initially stationary atom when the energy exceeds  $E_d$  is

$$\bar{E} = E_d \log \left( \frac{E}{E_d} \cdot \frac{4\mu^2}{M_1 M_2} \right) \quad (9)$$

The log term is very insensitive to  $E$  and ranges between about 3 and 15 for the values that are of most interest at present.

The ratio of (8) to (6), namely,

$$R_d = \log \frac{E}{E_d} \cdot \frac{4\mu^2}{M_1 M_2} \bigg/ \log \frac{E}{E^*} \quad (10)$$

represents the fraction of the energy dissipated in elastic collision that is expended in producing permanently displaced atoms. This ratio is also very insensitive to  $E$  and is about 0.5 for range of interest to us here.

(ii) **Excitation and Ionization of Electrons.** Let us first consider the case in which the energy parameter  $\epsilon$  (eqn. (1)) is large compared to the binding energy of the electrons in the material and in which the moving system can be regarded as a point charge. The Born approximation then can be used for treating the problem of excitation and ionization in keeping with the well-established developments<sup>4</sup> of Bohr, Bethe and others. The expression for the rate of dissipation may be written in the form

$$-\left(\frac{dE}{dx}\right)_e = \frac{4\pi z^2 e^4}{mv^2} N_0 Z_2 \log \frac{\epsilon}{B} \quad (11)$$

in which  $ze$  is the charge on the moving particle,  $Z_2$  is the number of electrons on the stationary atom and  $B$  is an energy parameter which is of the order of magnitude of the geometrical average of the ionization potentials of the various electrons in the atom. The other constants and parameters have the meanings given previously. Estimates of  $B$  may be made on purely theoretical grounds. However the best values for practical purposes are determined by experimental investigations of stopping power.

In the range of energy in which (11) is applicable the ratio of (11) and (6) is

$$\frac{(dE/dx)_e}{(dE/dx)_c} = \frac{z^2}{Z_1^2 Z_2} \cdot \frac{M_2}{m} \cdot \frac{\log \epsilon/B}{\log E/E^*} \equiv R \quad (12)$$

This reduces to

$$\frac{2M_2}{Z_2 m} \frac{\log \epsilon/B}{\log E/E^*} \quad (13)$$

if we assume  $z = Z_1$ . The ratio is insensitive to  $E$  and is of the order of  $10^8$  for typical cases. Thus the loss arising from electron excitation and ionization greatly overwhelms that arising from elastic encounters when  $\epsilon$  is sufficiently large.

Unfortunately, we have considerable interest in values of  $\epsilon$  that are of the same order of magnitude as the binding energy of the valence electrons in normal atoms so that eqn. (11) is not accurately applicable in the entire range. Moreover the moving particle need not be a point charge but may be a partly stripped atom whose total charge varies as the velocity decreases.

Let us consider for a moment the energy that is dissipated by the moving atom in exciting the electrons in a single shell of the stationary atoms. If the moving particle were a point charge the corresponding contribution to (11) would be

$$-\frac{dE}{dx} = \frac{2\pi z^2 e^4}{\epsilon} Z_i \log \frac{\epsilon}{B_i} \quad (14)$$

in which  $Z_i$  is the number of electrons per atom in the shell and  $B_i$  is an energy parameter characteristic of the shell. This expression is accurate only when  $\epsilon$  is very large compared to  $B_i$ . We note, however, that the function  $\log(\epsilon/B_i)/\epsilon$  rises to a peak when  $\epsilon = 2.71B_i$  and then drops to zero when  $\epsilon = B_i$ . We may expect that the true function governing the dissipation of energy by a single group of electrons exhibits a qualitatively similar behaviour when  $\epsilon$  becomes comparable to the excitation energy of the electrons in a given shell. That is, the dissipation of energy by the corresponding electrons attains its peak value when  $\epsilon$  is of the order of magnitude of the excitation energy of the electrons in the shell and then falls to zero as  $\epsilon$  approaches zero. Elementary reasoning shows that this decrease takes place transcendently if the moving particle is a heavy one so that it has sufficient energy to excite the bound electrons even when  $\epsilon$  is very much smaller than the excitation energy. Evidently a moving electron cannot excite a bound one when its value of  $\epsilon$  falls below the excitation energy since  $\epsilon$  is equal to its kinetic energy.

It will be sufficient for the following purposes to assume that the moving particle ceases to excite a given group of bound electrons when the parameter  $\epsilon$  drops to a value equal to  $E_e/8$ , where  $E_e$  is the excitation energy of the group of electrons. We shall adopt this assumption even when we are dealing with the excitation of the valence electrons in an insulator, in which case  $E_e$  will be taken to be the energy associated with the first absorption band of the bulk material. Evidently the lowest excitation energy of the entire aggregate of stationary atoms in the system will be associated with the valence electrons since these are the least tightly bound. Once  $\epsilon$  has decreased below the lowest possible value of  $E_e/8$ , the moving particle will be able to lose further energy only as a result of elastic collisions.

There is one interesting and important case in which an exception to the use of the rule formulated in the preceding section must be made, namely, that in which the system contains free electrons, that is, in the case of a metal. The problem of dissipation of energy by a gas of perfectly free electron can be solved in a simple, although approximate, manner by treating the moving

particle as if it were stationary and the electron gas moved past it. Those electrons which come within the range of interaction of the moving particle are elastically scattered in the reference frame in which that particle is regarded as stationary. Thus the energy loss can be derived by considering the elastic scattering of the free electrons by a centre of force. It is convenient to employ the Born formula (3) to evaluate the scattering cross-section in conjunction with the assumption that the potential of interaction is given by the Fermi-Thomas field for the neutral atom. The use of the field for the neutral atom is justified in the case of a slowly moving particle because of the screening effect of the conduction electrons. It is evident that the results obtained by this highly approximate procedure have no more than semi-quantitative value. Nevertheless, they provide an insight into the manner in which the conduction electrons contribute to the stopping power.

The momentum vectors of the electrons in the ideal free electron gas lie within the sphere of radius

$$p = \hbar \left( \frac{3nN_0}{8\pi} \right)^{1/2} \quad (15)$$

at the absolute zero of temperature. Here  $n$  is the number of free electrons per atom and  $N_0$ , as previously, is the density of atoms, and  $\hbar$  is Planck's constant. We shall designate the energy associated with the momentum (15) by  $\epsilon_0$ . The method of calculation described in the preceding paragraph leads to the following results—

$$(a) \ \epsilon \gg \epsilon_0: \quad -\frac{dE}{dx} = \frac{\pi Z_1^2 e^4}{\epsilon} N_0 n \left( \log \frac{\epsilon}{\epsilon_0} + 1.08 \right) \quad (16)$$

in which  $Z_1$  is the atomic number of the moving particle. For very large values of  $\epsilon$  this expression resembles (11) closely in form although the coefficient of the log term in (16) is just half that appearing in (11), presumably because of the absence of screening in (11).

$$(b) \ \epsilon \ll \epsilon_0: \quad -dE/dx = 12\pi N_0 n \epsilon A_0 Z_1^{3/2}; \quad A_0 = 76.8 a_0^2 \quad (17)$$

in which  $a_0$  is the Bohr radius. Eqn. (16) evidently is far more accurate than (17) since the various assumptions made are more justified when  $\epsilon$  is large compared with  $\epsilon_0$ . According to (17) the rate of dissipation falls linearly with  $\epsilon$  as this parameter approaches zero instead of in the transcendental manner characteristic of an insulator. This behaviour is a direct consequence of the fact that the free electrons may absorb energy from fields of arbitrary low frequencies.

(c)  $\epsilon \sim \epsilon_0$ . The rate of energy loss attains its maximum value when  $\epsilon$  is of the same order of magnitude as  $\epsilon_0$ .

In the range of  $\epsilon$  for which this parameter is of the same order of magnitude or greater than  $\epsilon_0$  the loss of energy through electron excitation overwhelms that arising from elastic collisions between the coulomb fields of the nuclei. The two become comparable only when  $\epsilon$  is considerably smaller than  $\epsilon_0$ . The ratio of (6) to (17) is

$$\frac{(dE/dx)_e}{(dE/dx)_i} = \frac{Z_1^{4/3} Z_2^2}{3n} \cdot \frac{m}{M_2} \cdot \frac{E_R^2}{\epsilon^2} \cdot \frac{\log(E/E^*)}{76.2} \quad (17a)$$

where  $E_R$  is the Rydberg energy. This ratio becomes large compared with unity when  $\epsilon$  becomes sufficiently small, showing that inelastic collisions eventually account for the major part of the energy loss when the moving particle becomes sufficiently slow, just as in insulating materials. For comparative purposes, it is interesting to note that this ratio is close to unity when  $\epsilon = E_R Z_1^{1/3} / 250n$ , in the case in which  $M_1 = M_2 = M$  and  $Z_1 = Z_2 = Z$ . This value of  $\epsilon$  ranges between about 0.1 eV and 10 eV for the

interesting materials of the periodic system, depending upon the values of  $Z$  and  $n$ .

The results of the preceding discussion may be conveniently summarized in the following way—

(1) As long as the energy parameter  $\epsilon$  of the moving particle is large compared with the excitation energy of the valence electrons, or with the parameter  $\epsilon_0$  for metals, the moving particle dissipates the greatest part of its energy in the excitation of electrons, the fraction being given by an expression of the type of (12).

(2) Electron excitation essentially ceases when  $\epsilon$  becomes sufficiently small and elastic collisions become predominant. It is convenient to assume that electron excitation stops abruptly when  $\epsilon$  decreases to a threshold value  $\epsilon_i$ . This threshold will be taken to be  $E_e/8$  in the case of an insulator, where  $E_e$  is the first excitation energy of the material. In the case of a metal it will be taken as the value of  $\epsilon$  for which (17a) is unity.

If the initial value of  $\epsilon$  for the particle is larger than  $\epsilon_i$  the energy which it expends in elastic collisions in being brought to rest may be expressed in the form,

$$E_c = \frac{M_1}{m} \left( \epsilon_i + \frac{(\epsilon - \epsilon_i)}{R} \right) \quad (18)$$

in which  $R$  is the ratio of the energy lost in electron excitation to that lost in elastic collisions when  $\epsilon$  is large.  $R$  is given by eqn. (12) for the case of a moving point charge and may be treated as nearly constant for a wide range of  $\epsilon$ . The second term on the right-hand side of (18) becomes comparable to the first only when  $\epsilon$  is of the order of 1000 times larger than  $\epsilon_i$  since  $R$  is of this order of magnitude in typical instances. Thus, when  $\epsilon_i$  is 1 eV, the second term will be comparable to the first only if  $\epsilon$  is about 1000 eV, which corresponds to an  $\alpha$ -particle with an energy near 8 MeV, or to a proton with an energy near 2 MeV.

Eqn. (18) evidently is replaced by

$$E_c = \frac{M_1}{m} \epsilon \quad (19)$$

when  $\epsilon$  is less than  $\epsilon_i$ .

**3. Behaviour of Knocked-on Atoms.** We have seen that the primary atom expends part of its energy in elastic collisions and part in exciting electrons. Of the first part, a fraction  $R_d$ , given by eqn. (10), produces displaced atoms. The average energy  $\bar{E}$  received by a displaced atom is given by eqn. (9). The secondary atoms invariably have a value of  $\epsilon$  that is below  $\epsilon_i$  so that all of their energy will be expended in elastic collisions. Since  $\bar{E}$  is between 3 and 15 times larger than  $E_d$  in typical cases, it follows that the secondary atoms may in turn eject tertiaries from the lattice. The secondary will dissipate about half its energy in exciting lattice vibrations and will, on the average, share its remaining energy equally with a tertiary because its speed is sufficiently low that the atoms will behave like rigid spheres. If after this collision the original secondary and the tertiary possess sufficient energy they may produce quarternary atoms. A simple analysis shows that the total number of atoms  $n_s$  displaced as the result of the production of a single secondary is approximately

$$n_s = \sqrt{\bar{E}/E_d} \quad (20)$$

Thus the total number of atoms displaced by the primary is

$$N = \frac{R_d E_c}{\bar{E}} n_s = \frac{R_d E_c}{\sqrt{\bar{E} E_d}} \quad (21)$$



We may conclude that one displaced atom is produced for each unit of energy of amount

$$\frac{\sqrt{\bar{E}E_d}}{R_d} \quad (22)$$

that is expended in elastic collisions. Since  $R_d$  is of the order of 0.5 and  $\bar{E}$  varies between about 3 and 15 times  $E_d$  in typical case, it follows that the energy unit (22) lies between  $3E_d$  and  $8E_d$ , or between 75 eV and 200 eV when  $E_d = 25$  eV. More definite values for particular cases will be given below.

**Displacements Produced by Charged Particles.** Let us consider the displacements produced in several typical materials by typical charged particles, namely, by a 5 MeV alpha-particle and by a 20 MeV proton. The materials to be considered are as follows—

Beryllium metal,	Diamond,	Silicon,
Graphite,	Aluminium,	Germanium.

Table I contains the relevant parameters for these materials. In the case of the metals beryllium and aluminium  $\epsilon_t$  is obtained by setting (17a) equal to unity. The values for diamond and graphite are judicious guesses based on the known optical properties, whereas the values for the semi-conductors silicon and germanium are based on the measured values of the energy gaps, based on measurement of electrical conductivity. Table II contains interesting quantities which appear in the calculation of the number of displacements  $N$  caused by 5 MeV  $\alpha$ -particles. The quantities in the first five columns, which depend slowly upon temperature, were treated as constants and evaluated for an  $\alpha$ -particle of intermediate energy, namely, 2.5 MeV. The values of  $\log \epsilon/B$  appearing in the third column is based upon the semi-empirical analysis of Livingston and Bethe.<sup>5</sup> In all cases except diamond,  $E_c$  (eqn. (18)) is composed mainly of the contribution from the second term. The penultimate column gives the reciprocal of the energy unit (22), which measures the amount of elastic energy expended on the average in producing one displacement. Table III contains similar data for the 20 MeV proton.  $E_d$  was taken to be 25 eV in all cases. The second term of eqn. (18) is even more important in this case than in that of the  $\alpha$ -particle.

The values of  $N$  in Table III, for 20 MeV protons, lie between 56 and 96 for the materials listed. A proton of this energy will penetrate through about 0.5 g. cm<sup>-2</sup>, or about 0.1 cm. for the materials listed. A layer of this thickness contains about  $5 \times 10^{21}$  atoms per square cm. Approximately 10 % of the atoms in such a layer would be displaced as a result of having a charge of one coulomb per cm.<sup>2</sup> in the form of 20 MeV protons fall upon it. An irradiation of this magnitude can be achieved with comparative ease.

The 5 MeV alpha-particles upon which Table II is based have a range of about 4 mg. cm.<sup>-2</sup>, or about  $10^{-3}$  cm. for the materials listed. Ten per cent. of the atoms in a layer of this thickness would be displaced as a result of having about  $10^{17}$   $\alpha$ -particles strike unit area. This degree of irradiation would be relatively difficult to achieve with a natural source of  $\alpha$ -particles since, to avoid self-absorption, it would be necessary to make the source about  $10^{-3}$  cm. thick and hence weigh no more than 5 mg. cm.<sup>-2</sup>. A source of this type would be required to possess a strength of at least one Curie to provide the required number of  $\alpha$ -particles in one year. Polonium has a sufficiently short half-life to meet the conditions. The desired intensity could be obtained relatively easily with a cyclotron.

<sup>5</sup> Livingston and Bethe, *Rev. Mod. Physics*, 1937, 9, 245.

TABLE I  
PARAMETERS FOR SEVERAL SOLIDS ( $E_d = 25$  eV)

	$Z_1$	$M_1$	$\epsilon_1$ (eV)
Beryllium metal ..	4	9	0.14
Graphite .. ..	6	12	0.5
Diamond .. ..	6	12	2.5
Aluminium metal ..	13	27	0.35
Silicon .. ..	14	28	0.14
Germanium ..	32	73	0.10

TABLE II  
NUMBER OF DISPLACEMENTS PRODUCED BY 5 MeV  $\alpha$ -PARTICLE  
( $\epsilon = 680$  eV)

	$\log E/E^*$	$R_d$	$\log \epsilon/B$	$R$ eqn. (12)	$\bar{E}$ (eV)	$E_c$ (KeV)	$R_d/\sqrt{EE_d}$ (eV <sup>-1</sup> )	$N$
Beryllium .	20	0.57	3.1	$1.29 \times 10^3$	282	4.8	$6.8 \times 10^{-3}$	33
Graphite .	20	0.56	3.0	1.10	280	8.2	6.7	55
Diamond .	20	0.56	3.0	1.10	280	23.0	6.7	154
Aluminium.	20	0.56	2.1	0.80	280	8.8	6.7	59
Silicon .	20	0.54	2.1	0.78	268	7.4	6.6	49
Germanium	20	0.50	1.4	0.59	248	9.2	6.4	59

TABLE III  
NUMBER OF DISPLACEMENTS PRODUCED BY 20 MeV PROTON ( $\epsilon = 11,000$  eV)

	$\log E/E^*$	$R_d$	$\log \epsilon/B$	$R$	$\bar{E}$ (eV)	$E_c$ (KeV)	$R_d/\sqrt{EE_d}$ (eV <sup>-1</sup> )	$N$
Beryllium .	21	0.56	6.2	$2.40 \times 10^3$	298	8.6	$6.5 \times 10^{-3}$	56
Graphite .	21	0.55	5.6	1.96	290	11.1	6.5	72
Diamond .	21	0.55	5.6	1.96	290	14.8	6.5	96
Aluminium.	20	0.52	4.5	1.63	273	12.9	6.3	79
Silicon .	20	0.55	4.3	1.58	272	12.9	6.7	81
Germanium	20	0.50	3.5	1.47	250	13.8	6.3	87

**Neutron Bombardment.** When neutrons pass through matter they may make knock-on collisions with the nuclei and hence produce displaced atoms. The energy transferred to the stationary atom is determined as a function of angle by eqn. (2). In this case  $M_1$  is the mass of the neutron and  $M_2$  the mass of the stationary atom. The average energy transferred to the atom is

$$\overline{\Delta E} = E \frac{2\mu^2}{M_1 M_2} \quad . \quad . \quad . \quad . \quad (23)$$

if the collision cross-section is isotropic.

Consider a 2 MeV neutron for illustrative purposes. The average energy that would be received by a knocked-on atom is given in Table IV for several cases, along with the corresponding value of  $\epsilon$  (eqn. (1)).

In all of the cases except hydrogen the knocked-on atom is in the range of velocity in which it dissipates its energy either by elastic encounters or by excitation of the valence electrons. Moreover, the values of  $\epsilon$  are sufficiently low that the fraction of the energy lost in elastic collisions becomes important only when  $\epsilon$  becomes less than  $\epsilon_e$ .

TABLE IV  
AVERAGE ENERGY TRANSMITTED TO AN ATOM  
BY A 2 MEV NEUTRON WHEN SCATTERING IS  
ISOTROPIC

Atom	$\overline{\Delta E}$ (KeV)	$\epsilon$ (eV)
H	1000	540
Be	360	21.7
C	280	12.7
Al	140	2.8
Ge	55	0.41

Table V contains values of the number  $N$  of atoms displaced in three interesting materials when they are employed to moderate neutrons, that is, to bring them to thermal equilibrium. It is assumed that a neutron having an initial energy of 2 MeV is brought to rest by a succession of elastic collisions in which it loses on the average in each collision the energy (23). The knocked-on atoms have successively lower energy. The quantity  $\xi$  in Table V is the fraction of energy the neutron loses in each collision and  $N_e$  is the number of collisions required to slow the neutron to thermal energy from 2 MeV.  $N_i$  is the number of collisions which the neutron must make before the knocked-on atom has an energy less than  $\epsilon_e$ . Until the neutron has been slowed to this extent the knocked-on atom will dissipate energy in exciting electrons before it is slowed to  $\epsilon_e$ . Subsequent knocked-on atoms will only have energy sufficient to produce elastic collisions.

It is interesting to note that the values of  $N$  in this case are between 10 and 100 times larger than those given in Tables II and III. Far less energy is expended in producing electron excitation because the knocked-on atoms begin with a much lower value of  $\epsilon$  than the primary  $\alpha$ -particle and proton considered in the previous section and hence make more efficient use of their energy in producing displacements.

**Fission Fragments.** Matter which contains fissionable material becomes the seat of a relatively large source of dissipation of energy when

exposed to neutrons since a pair of fission fragments possess about 160 MeV of kinetic energy. We shall focus attention on metallic uranium at first although the fissionable atoms might be suspended in another medium.

The fission process is usually asymmetrical, the values of  $\epsilon$  for a typical pair being 507 eV and 259 eV. These values are sufficiently large that the fragments are partly stripped of their electrons during the major part of the range, the degree of ionization decreasing as the fragments slow down. We shall assume that the rate of dissipation of energy as a result of electron excitation is given by an equation of the form,

$$\left(\frac{dE}{dx}\right)_e = \frac{4\pi e^4}{mv^2} Z_1^2 N_0 G(\epsilon) \quad (24)$$

in which  $Z_1$  is the atomic number of the fission fragment and  $G(\epsilon)$  is a linear function of  $\epsilon$  which has the value 16 when  $\epsilon$  is 500 eV and is zero when  $\epsilon$  is zero. This type of variation of  $G(\epsilon)$  appears to fit the observed behaviour of a fission fragment and included the influence of the decrease in degree of ionization with decreasing velocity.

TABLE V  
DISPLACEMENTS PRODUCED BY A 2 MEV NEUTRON IN  
BEING SLOWED TO THERMAL ENERGIES

Material	$\xi$	$N_e$	$N_i$	$N$
Beryllium metal	0.209	79	24.1	454
Graphite ..	0.158	104	21.1	1870
Aluminium ..	0.0724	240	14.1	6030

The use of (24) in combination with eqn. (6) for the rate of dissipation of energy in elastic collisions leads to the result that the fission fragments expend about 2.7 % of their energy in producing elastic collisions *during the part of the range in which electron excitation dominates* when brought to rest in metallic uranium. An additional amount of about 0.30 % is expended in this way after electron excitation ceases. About half of this energy is expended in producing displaced primary atoms. These atoms have an average energy of about 375 eV ( $\bar{E}$  in eqn. (9)) and produce three additional displaced atoms. By combining these quantities we find that the pair of fission fragments in metallic uranium produce 25,000 displaced atoms.

The number of displaced atoms is somewhat smaller if the fissionable material is embedded in a solid containing light atoms. Consider, for example, graphite; the quantity  $G(\epsilon)$  in (24) takes the initial value of 6 instead of 16 so that the rate of dissipation by electron excitation per atom is somewhat smaller. On the other hand the rate of dissipation by means of elastic collisions is also smaller because the quantity  $Z_2$  in the numerator of (6) is 6 instead of 92, which more than compensates for the fact that  $M_2$  in the denominator drops from 238 to 12. It is found that the fraction of energy of the fission fragment spent in producing elastic collisions during the period in which electron excitation predominates is 0.62 %, whereas the fraction spent after electron excitation ceases is 0.15 %. It follows that the pair of fission fragments produce about 8300 displaced atoms.

**Concluding Comments.** Burton<sup>1</sup> has already pointed out that effects which can be ascribed unmistakably to the influence of displaced

atoms have been observed in graphite and other metallic or near metallic substances. The discoloration of minerals and salts by radiations has been a topic for many years and the literature on this subject is large.<sup>5</sup> Lind has pointed out that most of the discoloration produced by the radiations of radioactive substances can be explained as a result of the production of electron excitation which evidently is a major source of energy dissipation even when the radiation consists of massive particles. In other words, the effects are similar to those produced by light, cathode rays and  $\gamma$ -rays. On the other hand, he and Bardwell<sup>6</sup> have found that the clear green coloration of diamond can be produced only by  $\alpha$ -rays, which indicates that, in this case at least, there are effects which are related directly to displaced atoms or to the heating effect which accompanies the displacement of atoms in elastic collisions.

#### NOTE ADDED IN PROOF:

Dr. S. Siegel of the Westinghouse Research Laboratory has kindly provided me with a copy of a manuscript which has bearing on the preceding paper and which is to be published in an early issue of *Physic. Rev.*

Siegel has found that a completely ordered specimen of  $\text{Cu}_3\text{Au}$  becomes essentially completely disordered after being exposed in the nuclear reactor at the Oak Ridge National Laboratory for a period of time sufficient to give a time-integral of flux of about  $3.3 \times 10^{19}$  neutrons per  $\text{cm}^2$  for neutrons having energies in excess of 50 KeV. The specimens were maintained at about  $40^\circ\text{C}$  during the irradiation. The probability that a copper or gold atom was struck by a neutron during this irradiation is of the order of  $2 \times 10^{-4}$ . Calculations of the type described in the preceding paper show that the number of atoms displaced should lie between 1 and 10 %, so that it is unlikely that the disordering is produced by direct displacement. Instead, it is much more probable that in this case the disordering is produced in the heated "wake" of the knocked-on particles. Since each copper atom which is knocked-on by a neutron receives of the order of 40 KeV on the average, it follows that the total energy imparted to the material during the irradiation is of the order of 8 eV per atom. A large fraction of this is released as thermal motion along the track of the knocked-on primary and secondary atoms, the local temperature being very high for a short period. On the average all parts of the alloy will be heated above the transition temperature for the order-disorder reaction and then rapidly quenched, leaving the material disordered.

In this connection, it is also interesting to note that K. Lark-Horovitz and co-workers<sup>7</sup> have produced significant changes in the resistivity of semi-conducting germanium by bombardment with 10 MeV deuterons. Similar effects have been observed by Davis, Johnson, Lark-Horovitz and Siegel<sup>8</sup> as a result of neutron bombardment. Since the conductivity of pure germanium may be influenced by agents which are present to the extent of one part per million, it is clear that the investigations of this type, like those on discoloration, provide a very sensitive test of radiation effects.

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<sup>5</sup> Lind and Bardwell, *J. Franklin Inst.*, 1923, **196**, 521.

<sup>7</sup> *Physic. Rev.*, 1948, **73**, 1256.

<sup>8</sup> *Physic. Rev.*, 1948, **74**, 1255.



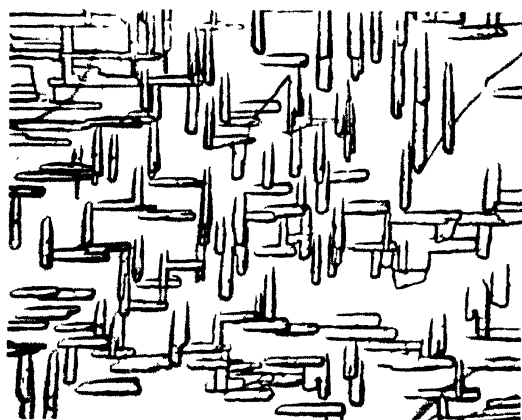


FIG. 1.-- Oriented overgrowth of hexaethylbenzene on anthracene.

## GENERAL DISCUSSION

**Dr. J. Willems (Krefeld)** (*partly communicated*): During the discussion on the paper presented by Mr. J. H. van der Merwe a question was asked whether sugar could be a substrate for oriented overgrowths of crystals of organic compounds. Sugar is an excellent substrate for such oriented deposits. Not only have oriented overgrowths on the cleavage plane of sugar been found with crystals of organic compounds containing strongly polar groups (e.g., pentachlorophenol<sup>1</sup> and carbon tetrabromide<sup>2</sup>), but even with organic compounds without such groups (e.g., aromatic hydrocarbons such as hexaethylbenzene and durol<sup>3</sup>).

A further question raised during the discussion was whether oriented overgrowth of sugar crystals upon a metal surface would be likely to occur. While oriented overgrowth of sugar crystals upon a metal surface has not yet been reported, such overgrowth of organic crystals upon a metal substrate can apparently occur.<sup>3</sup> But in such cases there exists the possibility of an oxygen layer on the surface of the metallic substrate.

In all cases so far described of oriented overgrowth of crystals of organic compounds, there is at least one component with one or more strongly polar groups. More recently it has been shown that the presence of strongly polar groups is not essential to the formation of oriented overgrowth of organic compounds. Thus oriented overgrowth of aromatic hydrocarbons (e.g., hexaethylbenzene on anthracene, Fig. 1) can be readily obtained.<sup>4</sup> In these cases the binding in the absorption process will be primarily through van der Waals' forces (dispersion forces).

**Mr. J. H. van der Merwe (Bristol)** (*communicated*): In reply to a question put privately by Dr. Willems, it is desirable to qualify the statement that it is an essential condition that the embryo must cover a flat region of the substrate *completely*. This is not strictly correct, since it is really only necessary that the growing edge of any one atomic layer must lag behind the edge of the atomic layer below it by a distance of the order of 20 atomic spacings; for then, because of their short-range interaction, dislocations cannot form spontaneously anywhere. When the gap between the edges closes up on a flat substrate, and dislocations form freely at its edge, the overgrowth will be at least two, but in general probably many more in some parts, atomic layers thick. Since the mobility of a dislocated film decreases with its thickness, a break away at this stage is unlikely to cause any change in the orientation.

Dr. Willems also kindly provided me with the following examples of oriented overgrowth:

(a)  $C_6Cl_6$  and  $C_6Cl_5CH_3$  on (110) surface of ZnS; the (001) plane and *b*-axes of the overgrowths being parallel to the (110) plane and  $[1\bar{1}0]$  direction of ZnS respectively.

(b) Hexaethylbenzene on (100) surface of NaCl.<sup>5</sup>

(c) Hexaethylbenzene on anthracene, anthracene on pyrene and phenanthrene on hexaethylbenzene.<sup>6</sup>

(d) Anthracene on pentachlorophenol, and naphthylamine and phenanthrene on benzidine.<sup>7</sup>

**Dr. J. Willems (Krefeld)** (*communicated*): As to the forces exerted by deposit units on the substrate (giving *W*) the theory outlined in the paper presented by

<sup>1</sup> Willems, *Naturwiss.*, 1943, **31**, 232.

<sup>2</sup> Willems (unpublished work).

<sup>3</sup> Willems, *Naturwiss.*, 1943, **31**, 208.

<sup>4</sup> Willems, *Naturwiss.*, 1949, **36**, 375.

<sup>5</sup> Willems and Giltges, *Naturwiss.*, 1946, **33**.

<sup>6</sup> Willems, *Naturwiss.*, 1948, **35**, 375.

<sup>7</sup> Brandstätter, *Mikrochem.*, 1947, **33**, 184.



Mr. J. H. v. d. Merwe is obviously in good agreement with chemical ideas recently developed in the field of oriented overgrowth.\*

Previous workers in this field came to the conclusion that preferred orientation can only be obtained, if the type of binding forces exerted by deposit units on each other is identical, or at least related, to the type of binding forces exerted by substrate units on each other.<sup>8</sup> At that time the cases of oriented overgrowth obtained preferably concerned cases where both partners were ionic, i.e., of identical type of binding. The experiments of some of the previous workers with organic partners were only successful with some few compounds of the phenol and the urea series. Experiments with other organic compounds, e.g., aromatic hydrocarbons, quinones, hexamethylenetetramine, etc., yielded no oriented overgrowth in spite of ideal geometrical conditions.

According to the recent chemical ideas mentioned above, the formation of oriented overgrowth is a typical topochemical process. Hence, in order that there shall be a definite orientation in a crystalline overgrowth of an organic compound on a crystalline substrate there must be formed a two-dimensional molecular compound between the units of the contact plane of the deposit and the corresponding units of the surface of the substrate.

These chemical considerations led to a systematical synthesis of oriented overgrowth of organic compounds on the base of the known types of organic molecular compounds, and the known lattice structures of the partners. Particularly partners were easily found for organic compounds such as aromatic hydrocarbons, quinones, etc., which did not yield oriented overgrowth in the experiments of previous workers. The oriented overgrowth of anthraquinone on NaCl (Fig. 2), corresponding to the molecular compounds of quinones with metal halides and anthracene on chloranil corresponding to the molecular compounds of aromatic hydrocarbons with quinones may be mentioned as typical examples.<sup>8</sup>

The oriented overgrowth of anthraquinone on antimony<sup>3 8</sup> shows that the type of binding forces exerted by the deposit units on each other need not be identical or related to the type of binding forces exerted by the substrate units on each other.

Soon the number of oriented overgrowths of organic compounds found on the base of these chemical considerations surpassed the number of the known oriented overgrowths of inorganic compounds.

**Mr. P. Woodward** (*Bristol*) said: In connection with the orientation of crystal growths by the substrate material, it might be of interest to show a few specimens of titanium nitride crystals, grown from the gaseous phase on tungsten filaments. Briefly, the process consists of the reduction of titanium tetrachloride vapour (using hydrogen) at high temperature in presence of nitrogen, the nitride being formed as a crystalline deposit on the filament. Titanium nitride forms very hard, golden yellow, cubic crystals, melting at over 3000° C; they can be grown at temperatures of about 1500° C.

Such crystals have been produced in the Inorganic Chemistry Laboratory here in Bristol. In our studies of the conditions under which they can be grown,<sup>9</sup> Dr. F. H. Pollard and I have found that the chemical nature of the filament has a marked influence on the appearance of the deposit of nitride obtained. Fig. 1 shows crystals of titanium nitride on tungsten as a randomly oriented growth of polycrystalline material. Fig. 2 shows crystals produced under exactly the same conditions, but using platinum as filament metal; the appearance is quite different. Fig. 3 shows the effect of using "aged" tungsten filaments (that is, filaments heated for several hours in a vacuum near the melting point so that preferred orientation of the crystallites along the filament axis occurs). It is obvious that the nitride crystals grow similarly with preferred orientation.

Here we have direct experimental evidence of the operation of the short-range forces discussed in van der Merwe's paper.

**Mr. P. R. Rowland** (*London*) said: Crystal growth is only one of many processes which can take place at the surfaces of lattices. The study of crystal surfaces must therefore rank as one of the central pillars of modern physical chemistry.

\* Willems, *Naturwiss.*, 1944, **32**, 324.

<sup>9</sup> Pollard and Woodward, *J. Chem. Soc.*, 1948, 1709.

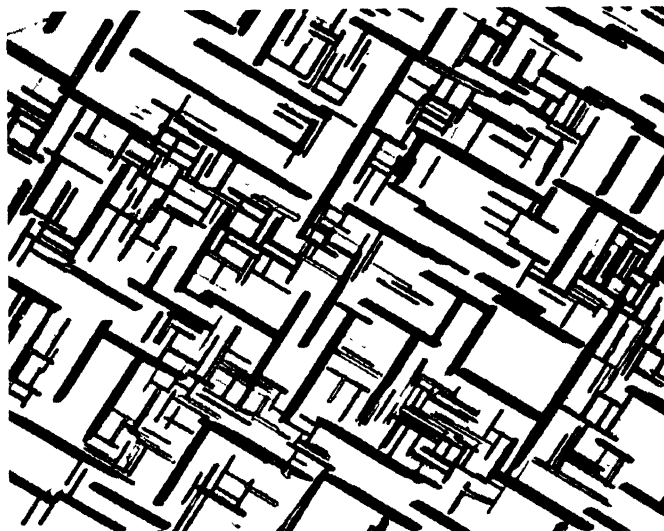


FIG. 2 ---Oriented overgrowth of anthraquinone on NaCl

(See Mr. P. Woodward's remarks on page 284)



FIG. 1.



FIG. 2.



FIG. 3.

Not only will the information obtained be of value in itself, but it may also give us clues on the structure and properties of solids and liquids themselves. For instance, Prof. Stranski's second paper indicates how surface structure may affect mechanical strength. In line with this view, the author, working under Prof. C. S. Gibson, has been studying the etching of spherical single crystals of copper by the halogens. The spheres, which were probably the purest copper single crystal material yet produced, were electrolytically polished and subjected to attack by the vapour of the halogen concerned at about 0.5 mm. pressure and 450° C. Under the conditions of the experiment, cuprous halide is sublimed off and the crystal is left with a brilliant etched surface. The micro-facets produced can be orientated fairly easily by the way in which they reflect light. Results have been obtained so far for  $I_2$  and  $Br_2$ . In both cases the facets which appear are (111), (012) and possibly (011). The following tentative explanations are offered.

(i) At 450° C these faces are the most stable and therefore appear automatically whenever copper is attacked.

(ii) The halogen attacks at steps on these faces and is therefore able to peel off the copper atoms layer by layer. This would be analogous to the reverse of crystal growth by step formation.

(iii) The cuprous halides at this temperature are probably analogous to  $\alpha$ -AgI, consisting of a body-centred cubic halide ion lattice with the interstitial positions occupied statistically by the metal ions which can move easily through the lattice. The (111) face of copper has hexagonal symmetry. The other face of copper which definitely appears is (012). The atoms in this are arranged at the points of an elongated hexagonal lattice. In both cases a rough fit can be obtained of the (111) face of the halide ion lattice if distortions of the type indicated by Dr. van der Merwe are permitted. Is it possible that the faces which appear are stabilized by a thin film of the halide so fitted? The copper could then move freely to the surface of the film and react with the halogen. Interesting speculations arise as to the thickness of the film, etc., but since its existence is itself a speculation this would be unwise at this stage.

It may be asked what the above experiments have to do with crystal growth. If (i) is the correct interpretation, then we have information on the stability of copper surfaces. If (ii) is the explanation, the method may yield information on the conditions at steps in crystal faces, whereas (iii) would be an interesting case of orientated overgrowth with the deposit adhering to the substrate more strongly than to further deposit and the copper rendered mobile over the surface. Further work is in progress on this and allied problems.

**Mr. H. E. E. Powers** (*London*) said: It has been reported how the form of certain crystals varied with the metal surface upon which they were formed. An even stronger case is that sucrose crystals, almost fully grown, suspended in a supersaturated syrup, when cooled in contact with Hyduminium, orient themselves in such a way that a very high proportion of the crystals set with their large plane face parallel to the metal, rather than in the completely erratic orientation attained in contact with iron. The result is seen in the mirror-like reflection and the silky feel of the slab ultimately removed from the Hyduminium surface as contrasted with the scattering of light and the "sharp tooth" roughness of that in contact with iron. This suggests a correspondence in the molecular lattice structure of sucrose and Hyduminium.

**Prof. A. Jullard** (*Brussels*) said: The attractive explanation of the action of phosphates proposed by Dr. Raistrick seems to hold well for  $CaCO_3$  precipitation and  $NaNO_3$  crystallization because one of the parameters of these crystals has a length of exactly 1.94 Å.

But the peculiar inhibiting action of Na hexametaphosphate must be related to something more than the geometrical properties of their molecules because this substance also acts as an inhibitor at concentrations of 2 p.p.m. in the precipitation of substances, such as  $BaCO_3$ ,  $Ag_2CO_3$ ,  $Ag_2S$ ,  $AgOH$ ,  $Ag_3AsO_4$ ,  $CaC_2O_4$ , etc.

This inhibiting action must be related to the great affinity of the large molecules of hexametaphosphate for the Ca, Ba and Ag ions, which affinity promotes the

adsorption of the hexametaphosphate and thus prevents the subsequent aggregation of the constituents of the crystal.

**Dr. A. F. Wells** (*I.C.I., Dyestuffs*) said: The importance of dimensional fit between substrate and oriented overgrowth has been demonstrated in many cases. It is a very different matter, however, to extend this idea to account for the inhibiting action of metaphosphates on the nucleation of calcium carbonate solutions. Even assuming that the distances between calcium ions in (0001) faces of very small nuclei of calcite correspond closely to oxygen-oxygen distances in metaphosphate chains or rings (the structures of which have not yet been determined), Dr. Raistrick's explanation could only account for inhibition of growth on the basal plane of calcite, i.e., for the development of thin plates. Also, as pointed out by Prof. Juliard, the action of sodium metaphosphate is not confined to calcium carbonate but also extends to other compounds with quite different crystal structures.

With regard to the more general question of normal as opposed to abnormal growth, it would seem that certain fundamental experiments in crystal growth have not yet been attempted. For example, before any experimental study of *relative* rates of growth on different crystal faces can be profitably carried out it is essential to develop an experimental technique which will result in equal amounts of growth on crystallographically equivalent faces. It would seem, for example, that experiments to grow perfect cubes (or geometrically perfect square sections of cubic crystals) should logically precede studies of growth in solutions of high supersaturation, a feature of which is the unpredictable and inexplicable variation in rate of growth on presumably equivalent faces of a cubic crystal.

**Mr. P. R. Rowland** (*London*) (*communicated*): Dr. A. F. Wells has pointed out that, according to Dr. Raistrick's theory, growth of calcium carbonate crystals would only be inhibited on one face by sodium metaphosphate and similar substances containing P-O-P chains, thus producing thin plates. However, neither Dr. Wells nor Dr. Raistrick appears to have followed up the implication in any detail. The following questions may be asked:

(a) Supposing thin plates are formed, what would be their thickness? Would it be of molecular dimensions? If so, could the plates be easily broken up to form a colloidal solution?

(b) Could a very thin nucleus grow in the circumstances? Might not the surface fields be so modified as to prevent growth?

(c) May not growth on the edges of the plates be prevented by stabilization of low index faces tending to heal the high index faces which Dr. Bunn supposes are present? Perhaps chains overlapping from the faces of the plates may also have some effect.

The test of the theory would appear to be in an exhaustive analysis of the nature of the supersaturated solution concerned to attempt to determine the size of the inhibited nuclei.

**Dr. B. Raistrick** (*Birmingham*) said: The structure

$$\begin{pmatrix} \text{HO} & & \text{O} & & \text{OH} \\ & \diagdown & & \diagup & \\ & \text{P} & & \text{P} & \\ & \diagup & & \diagdown & \\ \text{HO} & & \text{O} & & \text{OH} \end{pmatrix}$$

mentioned by Dr. Wells for hypophosphoric acid has been considered and rejected by Nylén and Stelling, and by Blaser and Halpern. To their reasons I would only add that this structure would give the phosphorous atoms nine electrons each in the outer valency shell, possibly causing the hypophosphates to be paramagnetic, whilst Bell and Sugden have shown them to be diamagnetic. It must also be pointed out that a compound of this structure would, on the basis of the hypothesis advanced, be equally as incapable of stabilizing the supersaturation of calcium carbonate solutions as will be the one that I propose.

The second point as to why the nucleus does not grow indefinitely to produce a thin sheet of large area is dealt with in my paper which gives in more detail the ideas advanced during the discussion.

**Dr. F. C. Frank** (*Bristol*) (*communicated*): Prof. Garner suggests that an added substance could inhibit growth by adsorption at "the reproducible point."

This is not quite correct, for, as was shown by Frenkel, there will be many such points along every step (this has been further elucidated by Burton and Cabrera, who refer to these points as kinks in their first contribution to this Discussion). Moreover, supposing all of these points are blocked, fresh ones can be formed with ease, requiring an activation energy which is only a fraction of the latent heat. On the other hand, adsorption along the length of the step will suffice for profound modification of the growth rate, while still requiring only a small quantity of adsorbed material. If there are  $N$  dislocations per unit area, and the lattice spacing is  $a$ , the fraction of all lattice points in a face which lie along steps connecting these dislocations is of the order  $aN^{\frac{1}{2}}$ —say, one in 3000 if for  $N$  we assume the conventional value for an annealed metal,  $10^8 \text{ cm.}^{-2}$ . Given a crystal of high perfection, with growth fronts based on one or a few dislocations, growth could be inhibited by an almost unmeasurable trace of adsorbate. This is on the assumption that the crystal is first treated with inhibitor before being submitted to supersaturated solution. If, on the other hand, growth is already actively proceeding, with a growth pyramid whose faces are vicinal by an angle  $\theta$  to the habit-face, the step-sites form a fraction  $\theta$  of all sites, for any small or moderate density of dislocations. The amount of adsorbate required to stop growth in this case is larger (e.g., if  $\theta = 10' = 0.3\%$ ).

The adsorption sites along the step-line are distinctively different adsorption sites from any others in the face (or in any other face of the same crystal). Especially for adsorbates of relatively complicated molecular geometry (such as dyestuffs or condensed phosphate ions), the adsorption energy at these step-sites can be very different from elsewhere, and, moreover, can be strongly dependent on the crystallographic direction of the step in the face.

If, now, adsorbed molecules each occupying  $n$  lattice-sites are adsorbed along the step-line, the "reproducible event" becomes the addition of  $n$  lattice units instead of 1, and demands the heat of desorption of the adsorbate molecule before it can take place (assuming the latter is rigid). A large value of  $n$  contributes to the efficiency of the inhibitor in two ways: by making this activation energy large and by making the adsorption specific and therefore economical. If the adsorbate consists of chains with some flexibility a complication is introduced. A certain amount of growth can occur by stages which do not involve desorption of the whole adsorbate molecule: but in time this will lead to the molecule becoming extended over the surface of the crystal, so as to give no play to its flexibility until it suffers a major readjustment in position. This, coupled with the fact that adsorption on the step-lines makes all faces, high-index faces included, into slow-growing faces, can account for the spherical form assumed by carbonate crystals whose growth is partially inhibited by metaphosphates, as described by Juliard.

One more point of interest which arises here is the apparently highly specific dependence upon agreement in lattice spacing between carbonate crystal and metaphosphate additive. It seems that not more than 1% or 2% discrepancy is needed for a marked decrease in effectiveness of inhibition. According to Frank and van der Merwe (see van der Merwe's paper in this Discussion) the "critical misfit" is 9% when the forces between adsorbate atoms and those between adsorbate and substrate atoms are equally strong. In this case the adsorbate atoms are covalently linked, but have mainly ionic attachment to the substrate. The cleavage properties of silicates show the greater strength of the former type of bonding and the fact that autunite cleaves like mica shows that the same relationship applies in phosphates. Hence it is to be expected that the critical misfit should be substantially less than the standard 9%.

**Dr. C. W. Bunn** (*I.C.I., Plastics*) said: The fact<sup>10</sup> that such widely divergent types of impurity as  $\text{K}_2\text{CrO}_4$  and the huge dye-molecule Brilliant Croceine 9B both affect the (011) faces of  $\text{KClO}_3$  is not necessarily to be regarded as evidence against the theory of habit modification (suggested by Royer and myself) which attributes the effects to a correspondence of lattice dimensions on particular crystal planes of the substances concerned. A correspondence of the lattice dimensions of a crystal plane of the impurity with multiples of the lattice

<sup>10</sup> Cp. Buckley, This Discussion.

dimensions on a particular plane of the modified crystal may be just as effective as a one-to-one correspondence; and until we know the lattice dimensions of Brilliant Croceine 9B we cannot say whether there is any correspondence with (011) of  $\text{KClO}_3$ . The same is true for all the other complex dyes which modify simple inorganic crystals. However, I do not want to push my own theory too far, and it may be that in some cases the adsorption of isolated impurity molecules is effective, while in others groups of impurity molecules are involved; it is only in the latter cases that lattice dimensions are likely to be important. It is perhaps significant that, as Buckley rightly points out, the cases in which a correspondence of lattice dimensions has been established are those in which a comparatively high concentration of impurity is required to effect habit modification; obviously it is precisely in these circumstances that groups of adsorbed impurity molecules are likely to be formed on the crystal faces, and their stability will depend on whether the lattice dimensions of the two substances correspond. On the other hand, the substances which are effective in very small concentration are often those with large molecules; in the first place, when the concentration of impurity is small, molecules may be adsorbed singly; and in the second place, a single large molecule, anchored on the surface by perhaps one polar group, constitutes as serious an obstruction to growth as a group of smaller molecules.

The examples in which a low concentration of a dye encourages one face, while a higher concentration encourages a different face, might be explained on these lines: at a low concentration the effect produced is due to adsorption of single molecules (the face affected being that on which isolated molecules are most stable), while at a higher concentration the effect is due to groups of adsorbed molecules formed on the face whose lattice dimensions are appropriate, and this may be a different type of face from that most affected by single molecules.

The examples in which impurity is built into the crystal on faces other than that which is most retarded seem disconcerting at first sight. But I think a reasonable explanation can be found if we consider the molecular mechanism of retardation. The retardation of growth by adsorbed impurity depends on two things—two-dimensional stability combined with three-dimensional instability: the impurity molecules attached to the surface form a stable complex with the surface molecules of the crystal, but if more solute packs round the adhering impurity, the three-dimensional complex is unstable, and being unstable has a higher solubility, and is therefore either not formed at all (i.e., more solute molecular will *not* build round the impurity) or if formed is soon redissolved; in either event stable growth cannot occur until the impurity is turned off the surface. When impurity is built into the crystal, this is because the impetus of growth is sufficient to cover the impurity with fresh solute in spite of the instability of the three-dimensional complex. How much impurity is built in depends on the degree of instability. Moderate instability means only moderate retardation accompanied by some inclusion of impurity; but great instability means maximum retardation with little if any inclusion of impurity. Thus the faces on which impurity is built in are not always those whose growth is most retarded; and the faces on which impurity is not built in are of two types—those whose growth is not affected at all because the impurity is not strongly adsorbed, and those whose growth is most retarded.

**Dr. S. Fordham** (*Stevenston, Ayrshire*) (*partly communicated*): Adsorption of dye need not always be strongest on the face which appears most developed in the modified crystal habit. Under conditions of high supersaturation, when growth occurs by the spreading of visible layers, the dyestuff might be most effective if adsorbed on to the advancing edges of the layers. The plane of the crystal actually adsorbing the dyestuff would then be normally, or at least steeply, inclined to the plane which is finally developed. In this particular instance, therefore, the face developed might not strongly adsorb the dyestuff in solution.

Even in the case of slow crystallization by unimolecular steps, it is not clear that there must exist a quantitative correlation between face development and the adsorption of dyestuff. We may assume that a dyestuff is adsorbed on a crystal by reason of its polar groups which become attached at specific points





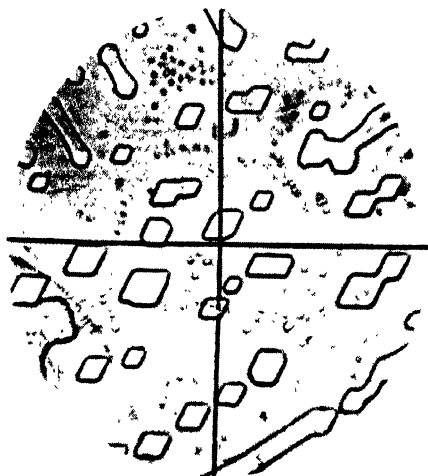


FIG. 1.

on the face or in vacant sites of the crystal. If this adsorption occurs at an advancing step, the polar groups may be adsorbed at two different levels and therefore effectively on an inclined plane of the crystal. Should this happen the dyestuff molecule is particularly well placed to affect the rate of crystallization. On the other hand, the amount of adsorption measured in the usual way would be expected to be greater for the inclined face than for that actually developed.

**Dr. J. Whetstone** (*Stevenston, Ayrshire*) said: In reply to Dr. S. Fordham's suggestion that adsorption of the dyestuff might be most effective on the advancing edges of the spreading layers during growth of the crystal under conditions of high supersaturation, it may be noted that, in ammonium nitrate crystals showing hour-glass inclusion of dyes, observations made of the pleochroism of the inclusions indicated that the plane in which the dye molecules must lie was parallel or nearly parallel with the (001) plane. Since habit modification was on the (010) plane, this supported Dr. Fordham's viewpoint.

Referring to the structural similarities between calcite and sodium hexameta-phosphate, Dr. Raistrick's observations were of the greatest interest in view of the fact that the *C* primitive translation of ammonium nitrate IV was also 4.96 Å, and sodium hexametaphosphate was known to modify the crystal habit of this salt to {001} "plates."

**Dr. S. Fordham** (*Stevenston, Ayrshire*) said: Some of the additions made to phases II and III of ammonium nitrate in the work of Prof. Hocart are known to slow down the rate of transition between these phases and phase IV. I would also draw attention to the metastability of dry ammonium nitrate of phase II followed by direct transition to phase IV at 50° C. In view of these known results, I would ask Prof. Hocart whether he considered that the stabilization observed was complete or whether it should not rather be regarded as increased metastability.

**Dr. J. Whetstone** (*Stevenston, Ayrshire*) said: Solid solution formation is apparently an essential preliminary to stabilization of a phase of ammonium nitrate outside its ordinary temperature range, and I should like to ask Prof. Hocart if he could elucidate the mechanism of stabilization of ammonium nitrate II by an insoluble material such as lead carbonate or silver sulphate.

**Prof. R. J. Hocart and Dr. J. C. Monier** (*Strasbourg*) said: Fig. 1 shows ammonium nitrate II oriented upon mica and stabilized at ordinary temperature by cesium nitrate (from a solution of ammonium nitrate saturated at 18° C and maintained at 50° C).

The experiments given in our paper did not take into account the possible double decompositions of salts which can take place when the impurity chosen has no ion in common with ammonium nitrate. Recent work involving such a process is being pursued by the authors as well as accurate determinations of the effective percentage of impurities added.

The expression stabilization has been employed in our paper to characterize phases I, II, III when brought into the field of "pure" ammonium nitrate IV. By such a term is meant: when studied at ordinary temperatures, the impure but "dry" phases I, II or III undergo no apparent change towards phase IV. In this respect, any one of those three phases is crystallographically inert (although thermodynamically unstable with regard to the decomposition products of ammonium nitrate).

Is this inertia phenomenon comparable to the well known metastable transition  $II \rightleftharpoons IV$  of "pure" and "dry" ammonium nitrate, at about 45° C, that is to say, within the domain of stable nitrate III? A suggested justification of this would be that in both cases the usual domains of stability are altered.

It seems advisable to limit the idea of domains of stability strictly to "pure" substances and merely to observe that impure phases I, II, III of ammonium nitrate are in false equilibrium or "inert" with respect to phase IV. New experiments would be necessary to determine the degrees of inertia.

**Dr. F. M. Lea** (*D.S.I.R., Watford*) said: The maximum pressure that can be exerted by unidirectional growth of a crystal, when the other ("free") faces are in contact with a solution not under pressure, is of considerable interest in

geology and many branches of technology. It is of interest, therefore, to enquire whether, on theoretical grounds, any limit can be set to the applicability of the equation

$$PV(\text{solid}) = RT \log c/c_s,$$

given by Prof. Correns, apart from any limitations which may arise from the influence of surface forces in determining whether the solution can continue to gain access to the stressed faces. As the degree of supersaturation rises an increasing tendency must exist for deposition to occur on the "free" faces of the crystal rather than on the end faces growing under restraint. Pressure on the end faces causes a very slight increase in solubility of the "free" faces but it is of another order of magnitude, being given, as Williamson<sup>11</sup> and Goranson<sup>12</sup> have shown, by the equation,

$$\frac{P^2}{E} \cdot V(\text{solid}) = RT \log c/c_s,$$

where  $P$  is the pressure on the stressed face and  $E$  the elasticity of the crystal. The problem, therefore, is to determine at what value of  $c/c_s$  the "free" face will commence to grow rather than further deposition occur on the end faces bearing the unidirectional stress. Present theories of crystal growth do not appear to explain why certain faces of a crystal should continue to grow preferentially against a progressively increasing pressure. Further study of this phenomenon may, it is suggested, throw further light on the factors which determine the relative rates of growth of different crystal faces.

**Prof. C. W. Correns** (*Göttingen*) (*communicated*): If a crystal grows under stress the unstressed free faces also grow if the pressure is small, i.e., as realized under experimental conditions and also observed in our measurements. The data for alum are given in Fig. 1 and Table I. From the equations of Dr. Lea and myself, it follows that the saturation pressure for the free and the stressed

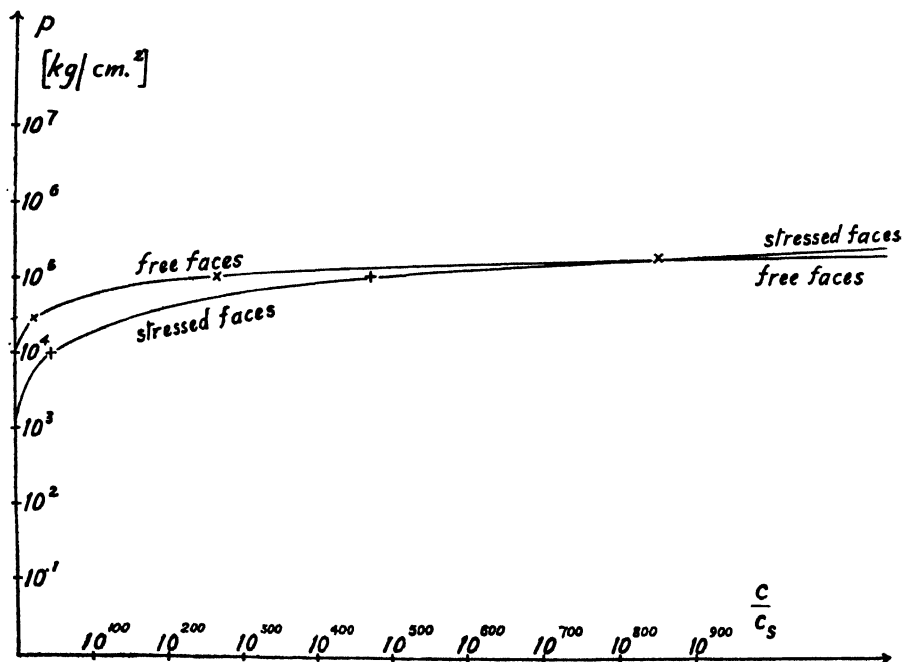


FIG. 1.

<sup>11</sup> Williamson, *Physic., Rev.*, 1917, **10**, 275.

<sup>12</sup> Goranson, *J. Chem. Physics*, 1940, **8**, 323.

faces is then the same when the pressure equals the elastic modulus  $E$ . (This is the case with alum at 180,600 kg./cm.<sup>2</sup>.) If the pressure is smaller the supersaturation is larger for the free faces than for the pressed ones. At pressures which are higher than this, it is the other way round. At these very high pressures the free faces would no longer grow but only the pressed faces.

The problem put forward by Dr. Lea can be answered in the following way. With a given experimentally possible supersaturation the pressed faces only grow until the pressure, corresponding to my equation, is reached while the free faces grow until the much higher pressure, corresponding to the equation given by Lea, is attained. Only with extremely large supersaturations, which are for alum larger than  $10^{854}$ , do the free faces cease to grow at smaller pressures than the pressed faces. With rock-forming minerals too it can be estimated that in nature the free faces will always grow even if one allows for hydrostatic pressure.

TABLE I

$P(\text{kg./cm.}^2)$	$c/c_s$ for the stressed faces	$c/c_s$ for the free faces
10	1.1	1.000005
31.6	1.4	1.00006
63.2	2.0	1.00025
100	3.0	1.0006
1,000	$5.3 \times 10^4$	1.06
10,000	$10^{47}$	$5.3 \times 10^8$
30,000	$10^{148}$	$10^{24}$
100,000	$10^{473}$	$10^{269}$
180,600	$10^{854}$	$10^{854}$
1000,000	$10^{4730}$	$10^{26900}$

## IV. MINERAL SYNTHESIS AND TECHNICAL ASPECTS

### Introductory Paper

By DR. F. A. BANNISTER

Artificial minerals have so far been made by processes essentially similar to those which have operated on a much larger scale in nature. Some of the conditions which influence crystal growth can now be controlled satisfactorily in the laboratory, but the pressures and temperatures and above all the time available for a particular experiment are limiting factors. Numerous attempts in the past to produce minerals identical in properties and composition with those naturally occurring as constituents of rocks and ores have had as their chief object the comparison of the laboratory conditions of formation with their mode of natural origin (paragenesis). It is chiefly within the last twenty-five years that it has been possible not only to grow large crystals but ones that are at least optically perfect.

Crystals can be grown by :

- (1) Cooling or evaporation of a saturated solution,
- (2) Condensation of a vapour,
- (3) Freezing of a liquid,
- (4) Applying heat and pressure to a dry melt,
- (5) Applying heat and pressure to a solid system containing water,
- (6) Reactions involving ionic diffusion below the melting point.

These methods are illustrated by the papers to be given in this section. Barrer reviews their application to the growth of crystalline silicates and compares the production of rock-forming minerals from natural magmas. Whereas artificial zeolite and clay minerals can be formed only by the hydrothermal method (5), quartz and the feldspars, orthoclase  $\text{KAlSi}_3\text{O}_8$ , and albite  $\text{NaAlSi}_3\text{O}_8$ , have also been produced by methods (3) and (4).

The hydrothermal method which has been used extensively for over a century to produce minerals and also to study their alteration products makes use of a sealed container, bomb or autoclave. It is supposed that conditions inside the enclosure resemble those that produced crystals in igneous rocks formerly subjected to high temperature and pressure. We can, of course, only witness the end products of these reactions even in the laboratory and the mechanism of hydrothermal crystal growth cannot so far be directly observed. We possess two great advantages in the laboratory: a knowledge of what and how much we put into the reaction chamber, and control of temperature and pressure. The contents of an autoclave can be subjected to a temperature gradient or heated under isothermal conditions. Water vapour above the critical point can dissolve silica and redeposit it as the least soluble form of quartz. A polycrystalline crust is first laid down upon the walls of the autoclave followed by single crystal growth upon a seed cut from a natural crystal.

Accounts of both variations of the hydrothermal method applied to the synthesis of quartz are given by Van Praagh, Thomas and the Woosters. These investigators have developed the isothermal technique and give details of modifications to Nacken's original method. Van Praagh's work is concerned chiefly with a study of the devitrification products of the transparent silica glass (vitreosil) used as raw material. Thomas and the Woosters have had as their main objective the production of artificial quartz suitable for the manufacture of oscillator plates. They have encountered one serious disadvantage. If the autoclave is charged with more than a given quantity of silica glass then devitrification can take place before the glass has all dissolved. Consequently five successive deposits each nearly 1 mm. thick upon the original seed crystal are necessary to obtain the required thickness. The total duration of the 5 mm. growth is 90 hr., including 25 hr. taken in heating the autoclave each time to 360° C. This corresponds to an increment of 100 mg./cm.<sup>2</sup> in one experiment of 18 hr. and cannot be exceeded if the growth is to be a single crystal and sufficiently free from flaws to serve as an oscillator.

An interesting speculation is whether quartz crystals grown naturally have comparable rates of growth. Should we ever possess a method of dating different levels of growth within a naturally occurring crystal it may prove that quartz crystals took no longer to grow in a mineral vein than they do in an autoclave. Another interesting question is whether the hydrothermal growth of crystals follows the principles which are now emerging from the theoretical and experimental studies described in previous sections. How far is single crystal growth of quartz in an autoclave dependent upon the presence of faces with high indices (Bunn, Part I) and what part is played by dislocations?

Wyart has applied the hydrothermal method of synthesis to minerals from their amorphous oxide constituents, and has produced cristobalite, analcime, kalsilite and orthoclase as well as quartz. He has made a study of the whole series  $\text{Na}_x\text{K}_{1-x}\text{AlSiO}_4$  with the object of specifying the limits of stability of nepheline ( $\text{Na}_3\text{KAl}_4\text{Si}_4\text{O}_{16}$ ) and kalsilite ( $\text{KAlSiO}_4$ ). The work of Michel-Lévy and Wyart deals not only with the hydrothermal synthesis of minerals in autoclaves but also with the products of gaseous explosions in which pressures of 50,000 kg./cm.<sup>2</sup> and temperatures of 30,000° C are attained for a few millionths of a second. This recalls the work of Sir Charles Parsons who thirty years ago used gunpowder explosions and attained similar temperatures and pressures in a series of unsuccessful and expensive attempts to make artificial diamonds. Michel-Lévy mixes samples of various powdered materials, including minerals and rocks, with explosives and produces

momentarily the high-pressure and temperature conditions that operated, for instance, in the formation of well-crystallized granite in the earth's crust. (The pressure exerted by the weight of overlying rocks at a depth of 10 km., i.e., 6.4 miles, assuming an average density of  $2.7 \text{ g./cm.}^3$  for the granitic layer, is  $2700 \text{ kg./cm.}^2$  and at that depth in the absence of all volcanic disturbance the temperature probably approaches  $350^\circ \text{ C.}$ ) The explosions produce glassy spherules mostly  $< 1 \mu$  diam., that, on annealing at temperatures up to  $850^\circ \text{ C.}$ , are converted to small but well-formed crystals of quartz ( $0.25$  to  $1 \text{ mm.}$ ), the feldspar minerals, biotite flakes, magnetite, etc.

Wyart points out that the relatively small quantities of crystalline solids formed in some of these experiments can be identified with certainty only by X-ray diffraction methods. Chemical and spectrographic techniques are of course as essential as ever they were in detecting and measuring the quantities of the elements present in a material. The majority of transparent and many opaque crystalline solids can be identified by their optical properties in transmitted or reflected polarized light providing that the crystal size does not approach too nearly or fall below  $1 \mu$ . It is now possible with X-ray technique to determine the crystalline phases present and in some instances their relative proportions, not only for minute test specimens but also for the fine-grained ( $< 1 \mu$ ) constituents of clays, metals, ceramics, cements and many other crystalline and partially crystalline materials. All these methods are of importance for the study of crystal growth particularly under hydrothermal and pneumatolytic conditions.

The study of crystal growth in a complex system like Portland cement or a blast-furnace slag demands first of all an investigation of the component phases, their properties and genesis. Of paramount importance is a knowledge of the crystal structure from which can often be deduced at least approximately the melting point, coefficient of expansion, cleavage, hardness and dielectric constant. Crystal structure analyses of calcium silicates and aluminates and other cement minerals have been hindered by the difficulty of preparing single crystals of some of the phases. Powder photographs do not suffice except for those with high symmetry. Techniques for the synthesis and growth of the low symmetry crystals have been developed at the Building Research Station and are described by Lea and Nurse.

Control of crystal habit although imperfectly understood has many practical applications. Lea and Nurse describe recent work on the setting of plaster of Paris. The usual radiating growths of gypsum needles, elongated parallel to  $[001]$ , in aqueous solutions can be modified, by the addition of sodium citrate, to short prisms tabular to  $(010)$ , so reducing the expansion of the plaster on setting. Control of crystal size can be just as important in refractory materials as in metals. Work carried out by the British Refractory Research Association shows that sintering of a magnesia brick at  $1600^\circ \text{ C.}$  increases the size of the periclase crystals with diminution of pore-space, thus improving mechanical properties. The well-known examples of the production of wollastonite (m.p.  $1540^\circ \text{ C.}$ ) from the interaction of lime and silica at  $700^\circ \text{ C.}$  and of forsterite (m.p.  $1890^\circ \text{ C.}$ ) from magnesia and silica at  $620^\circ \text{ C.}$  show how important reactions involving ionic diffusion below the melting point can be in the manufacture of refractories, ceramics and many building materials as well as in the thermal metamorphism of rocks.

The growth of large crystals from aqueous solutions still remains more an art than a science. Holden's work in the Bell Telephone Laboratories emphasizes the importance of the stirring conditions. Maximum deposition of crystal nuclei should be at the crystal surfaces and this is achieved by moving the crystals bodily through the solution and frequently reversing their direction of travel to prevent the liquid rotating with the crystals.

Details of how to grow crystals of different substances vary of course with solubilities and the type of habit modification that may be necessary. It is noteworthy that both Jaffé and Robinson who have worked independently on the growth of large crystals of ammonium dihydrogen phosphate for telecommunication systems use substantially similar technique and add the same salts to modify crystal habit. Each substance has its tolerable growth rate above which "veils," i.e., threadlike inclusions of mother liquor, begin to be included in the growing crystal. Holden attributes these to inequalities of supersaturation over various areas of a single face. Similar crystal outlines or "ghosts" are sometimes seen in naturally occurring crystals like quartz and are due to interruptions in crystal growth.

The growth of crystals from melts described by Stockbarger and Menzies has become one of the most important methods for the preparation of optically perfect materials. It is now possible to produce colourless crystals of fluorspar in large quantities, whereas the naturally occurring mineral is relatively scarce and usually restricted in crystal size. Large single crystals of sodium nitrate can be induced to solidify from a melt in contact with a sheet of mica that acts as a 'template' for the sodium atoms. Crystals grown from the melt approach the ideally perfect crystal of the X-ray crystallographer, whereas those grown from solution are more often mosaic in type, their growth, as Fordham suggests, depending on the propagation of dislocations. Knowledge of the influence of impurities on the perfection of crystals—grown from the melt—and upon their outer form should be of assistance in formulating the general principles of growth of ionic crystals.

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## THE PRODUCTION OF LARGE ARTIFICIAL FLUORITE CRYSTALS

BY DONALD C. STOCKBARGER

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This paper presents a condensed summary of the research and accomplishments in the development of methods and means for the growth of artificial optical fluorite at the Massachusetts Institute of Technology during the last war.<sup>1</sup> It touches lightly on some of the earlier studies, begun in the middle 1920s,<sup>2</sup> but does not include post-war improvements.

The work was done for the National Defence Research Committee, U.S.A.,<sup>3</sup> with a view to supplying optical material needed for highly corrected aerial camera objectives and the like. Altogether some 1500 crystal castings had been grown from the melt, ranging up to 6 in. diam., and several of them had been successfully incorporated in large instruments at the close of the war.

**Historical.**—It is practically impossible to trace the history of artificial fluorite research because of the paucity of published accounts. Few workers,

<sup>1</sup> O.S.R.D. Report No. 4690.

<sup>2</sup> Stockbarger, *J. Opt. Soc. Amer.*, 1927, **14**, 488; *Amer. Miner.*, 1927, **12**, 26.

<sup>3</sup> N.D.R.C. Contract OEMsr-45 with Massachusetts Institute of Technology.

it may safely be assumed, were sufficiently encouraged to continue their efforts and still fewer got so far as to describe their unpromising results. Judging by the meagre evidence found in the literature, most of the scientists must have encountered unwittingly the principal obstacle to direct crystallization of alkaline earth halides from the melt, viz., hydrolysis,<sup>4</sup> and so turned to methods of precipitation.<sup>5 6 7</sup>

In the middle 1930s, however, one laboratory resumed its earlier endeavours<sup>2</sup> hopeful that practical advantage could be taken of its apparatus and methods for growing optical lithium fluoride.<sup>8 9</sup> It succeeded in making single air-grown fluorite castings, 1½ in. diam., which had good cleavage but poor optical properties.\* From a review of this and other accumulated experience, a key to the solution was seen to lie in more efficient utilization of those factors which had already been helpful, viz., (a) care in salt preparation, (b) minimization of hydrolytic contamination and (c) temperature-gradient control.<sup>10</sup> A useful plan was formulated and put in practice, on a very small scale, in 1940. Immediate success led promptly to the government contract which enabled the laboratory to expand and improve the apparatus and procedures for growing optical fluorite crystals of practical dimensions for the first time.

**CaF<sub>2</sub> Stock.**—Calcium fluoride, suitable for conversion into artificial optical fluorite, was obtained from natural fluorspar and by synthesis in the laboratory. To be useful, it had to be relatively free from such impurities as silicates, sulphides, sulphates and carbonates.

Only colourless semi-optical fluorspar was found wholly satisfactory. Occasionally it was available in large pieces whose only noticeable defects were cracks. More often it had to be prepared through dissection and manual removal of all visible specks of foreign minerals and all portions exhibiting cloudiness or colour, a process which brought the cost up to many dollars per pound.

The spar was located principally by a special field survey group<sup>11</sup> and was shipped to the laboratory in lots of several tons by agents assigned to work in the mining districts. It was then sorted, broken if necessary and routed to a specially trained staff of stock pickers for dissection and segregation. Following preliminary visual grading, to be verified later in crystallization tests, the most hopeful accumulations were inspected, crushed, washed in cool water, rinsed with alcohol or other highly volatile solvent and dried at room temperature in a stream of filtered air. Here as always, in handling material to be crystallized from the melt, great care was exercised to avoid contamination. The importance of this was emphasized by the ruin of one lot of spar through washing with hot water and drying over a steam bath.

Hydrolysis was exceedingly troublesome. It became detectable below 100°C and increased rapidly with rising temperature to a disastrous magnitude far below the melting point. It very evidently contaminated

<sup>4</sup> Fremy, *Ann. Chim. Phys.*, 1856, **47**, 5.

<sup>5</sup> Mellor, *Comprehensive Treatise on Inorganic and Theoretical Chemistry* (Longmans and Co.), Vol. III.

<sup>6</sup> Friend, *Text-book of Inorganic Chemistry* (Charles Griffin and Co., Ltd.), Vol. III.

<sup>7</sup> Zekhnovizer, *J. Physic. Chem., U.S.S.R.*, 1937, **10**, 88.

<sup>8</sup> Stockbarger, *R.S.I.*, 1936, **7**, 133.

<sup>9</sup> Stockbarger, *This Discussion*.

<sup>10</sup> Stockbarger, *R.S.I.*, 1939, **10**, 205.

<sup>11</sup> N.D.R.C. Contract OEMsr-563 with Princeton University.

\* Much of the equipment used in this early work was purchased with funds granted by the Rumford Committee of the American Academy of Arts and Sciences.



the salt within the furnace long before the unavoidable adsorbed water was volatilized by heating under reduced pressure. Therefore it was advantageous, although not always necessary, to introduce a scavenger prior to fusion.\*

The most promising scavenging agent studied during the war was  $\text{PbF}_2$ .† It could be made to eliminate objectionable impurities arising not only from water but also from undetected traces of certain associated minerals such as calcite. Furthermore it possessed the advantages of relatively low boiling point and, apparently, easy rejection during the crystallization. The concentration of lead in the crystal was found spectroscopically to be less than one part per million in most instances and it was generally too low to have much, if any, ill effects on spectral transmissions and refractive indices.

The scavenger was thoroughly mixed with the  $\text{CaF}_2$  before the crucible was loaded. A surprisingly large proportion, several per cent., was usually required because the efficiency depended on the pre-fusion heating whose optimum course was not ascertainable under the circumstances and conditions obtaining at the time. Clearly there was room for improvement and so the use of  $\text{PbF}_2$  was regarded primarily as a wartime expedient.

**Synthesis.**—The synthesis of alkaline earth halides had always been attended by difficulty and became no exception during the wartime research on calcium fluoride. Although the material made by the laboratory was shown spectroscopically to be equal or superior to useful fluor spar in electropositive element content, none of it was ever successfully converted into crystals suitable for optical use even after the addition of  $\text{PbF}_2$ . It now seems likely, however, that at least a part of the failure may be attributed to improper crystallization conditions for, as became evident later, neither the furnace design nor the freezing control was nearly perfected at the time of the chemical work.

Since there is a possibility that simple synthetic  $\text{CaF}_2$  will eventually become useful, one of the best preparation procedures is presented. Briefly, it consisted in (a) converting selected non-optical calcite crystals into  $\text{Ca}(\text{NO}_3)_2$ , (b) treating a dilute solution of the nitrate with an excess of  $\text{NH}_4\text{OH}$  and  $\text{O}_2$  to remove magnesium and iron, (c) diluting further, after filtering, and precipitating  $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$  by adding dilute  $\text{H}_2\text{SO}_4$  solution, (d) converting the sulphate into carbonate by adding  $(\text{NH}_4)_2\text{CO}_3$  solution, (e) converting the  $\text{CaCO}_3$  into  $\text{Ca}(\text{NO}_3)_2$  by adding dilute  $\text{HNO}_3$ , (f) reconverting the nitrate into carbonate after filtering and diluting the solution, (g) reconverting the carbonate into 0.1 N nitrate solution, (h) adding the nitrate solution to 1 N HF solution at a rate of 1 l./hr., stopping while the solution was still acid, (i) washing the  $\text{CaF}_2$  with dilute HF solution and (j) drying the salt at room temperature in a low-pressure desiccator. It is to be understood that all precipitated products were washed repeatedly. Large volumes of liquid were used generally and their temperatures were kept elevated when appropriate. All chemical reagents were of tested purity and the HF was distilled one or more times. It also goes without saying that the utensils and filter paper were selected with due regard to avoidance of contamination.

Another, and presumably complex, synthetic material showed considerably more promise of being useful for growing fluorite. It was made quite simply by treating an aqueous suspension of finely precipitated  $\text{CaCO}_3$  and  $\text{PbCO}_3$

\* The idea of scavenging was conceived and applied, although not reported, during the LiF research.<sup>8</sup>

† Stockbarger and Blanchard, U.S. Pat. (applied for).

with an excess of HF solution in a lead-lined vessel and drying the product overnight at a moderate temperature. The procedure was susceptible of improvement but was neither developed nor employed during the war beyond the point of supplying sufficient stock for making a few crystals for test purposes.

All synthetic  $\text{CaF}_2$  materials possessed the inherent defect of relatively low density on account of their dendritic character, and therefore were inconvenient to use. They had to be pre-shrunk mechanically or thermally in order to permit filling the crucible with melt.

**Furnaces.**—The preferred furnace was one of several "vacuum"-type contrivances built especially for growing fluorite. Since it has been described in the references,<sup>1-9</sup> only its salient features need to be reviewed here.

In its thermal design, this furnace was similar to that originally developed for growing optical lithium fluoride<sup>8</sup> and later modified to produce large single, although optically useless, calcium fluoride crystals. It provided a suitably high temperature-gradient region, through which the crucible was lowered, to facilitate the purification naturally accompanying solidification of the melt and it contained remotely controlled means for determining whether or not the freezing was occurring at the most favourable level.

The heating elements were made of graphite which had been carefully selected with regard to chemical purity and mechanical strength. They received electric power via graphite rods from large water-cooled, mica-insulated, copper electrodes passing through the housing base plate which was also water-cooled. They were designed with care to avoid chilling of connections and consequent undesirable thermal field distortion. Their heat insulation was provided by sheet molybdenum baffles.

The water-cooled housing was sufficiently tight to permit evacuation to a small fraction of a micron after all heated internal parts had been thoroughly outgassed. This feature was necessary to exclude water vapour which would have caused hydrolysis of the  $\text{CaF}_2$ , thus contaminating the melt, and to prevent the oxidation of the hot graphite, tungsten and molybdenum parts.

Two mercury-vapour condensation pumps were used in series with a demountable vapour trap, chilled for safety by liquid nitrogen, connecting them to the mechanical pump. The trap was necessary only when scavenging was employed.

The thin wall, conical-bottom crucible was turned on the lathe from a graphite rod which was required to be dense, nearly physically perfect, and, moreover, devoid of deleterious chemical impurities which could not be removed through leaching with molten  $\text{CaF}_2$ .

Temperature regulation depended solely on a 90 KVA laboratory line voltage controller, developed especially for the purpose, capable of holding the input constant within a small fraction of 1 %. Ambient temperature fluctuations were, of course, without noticeable effect.

**Furnace Operation.**—Furnace operation was of such great influence on crystal quality that the topic requires consideration in some detail. Selected fluorspar is assumed to have been used with an appropriate proportion of  $\text{PbF}_2$  in the following hypothetical example.

The furnace housing was first sealed and evacuated to  $0.1 \mu$  pressure, for instance, to verify the tightness of all joints. The elevator was run up until the apex of the crucible bottom was above the gradient baffle. The heater power was then turned on gradually but fairly rapidly until the gas pressure rose to about  $10 \mu$  and was thereafter increased in sufficiently small steps to prevent the copiously evolved gas from raising the pressure much until the  $\text{CaF}_2$  melting point was neared. The heating process usually

consumed several hours and could not be hurried without danger of disastrous contamination of the melt. When the  $\text{CaF}_2$  temperature was about  $1000^\circ\text{C}$  (estimated), the scavenging operation was begun by turning up the power relatively rapidly so that the melting point was reached within a quarter-hour or so. During this period the gas evolution was violent and the vapour trap collected an appreciable quantity, perhaps 50 ml., of material, which was found to be highly corrosive and to have a very disagreeable odour after having been allowed to melt. Following the fusion, the gas pressure settled down almost immediately and remained below  $0.1\ \mu$  unless purified neutral gas was being streamed through the furnace to reduce the entrance of mercury vapour.

Complete melting was verified by means of the depth sound, i.e., freezing level probe, after which the elevator was lowered to place the crucible apex a little below the gradient baffle level. The power was then slowly reduced manually until the freezing level rose, for example, to the baffle height. The elevator motor could be turned on at this time to start the travel of the crucible down through the baffle at a speed of  $1/25$  in./hr.

Throughout the crystallization occasional depth soundings were made to determine whether power adjustments were required to maintain the freezing within the region of maximum purification. As soon as the soundings showed that all of the melt had frozen, the elevator motor, heating power and pumps were often shut off at once. If the crucible was very large, however, the heating power was reduced gradually in about 2 hr. before the pumps were stopped.

Much valuable information could be had from simple inspection of the crystal after it had been removed by turning the crucible upside down on a pile of cotton wool. If the casting was rose-coloured, experience suggested that a minute amount of air had leaked into the housing. If it was purple, contamination might have come from the graphite. If it scattered light generally from within, the freezing level had not been held fixed at the optimum height and/or the spar had not been picked carefully enough. If it scattered light directionally from its  $111$  planes, scavenging had been inadequate.

**Annealing.**—Newly grown crystals were usually so badly filled with strains that they could not be sawed and worked in the optical shop without cracking. They were greatly improved in this respect by heating slowly to about  $800^\circ\text{C}$  in a nearly gradient-free, evacuated furnace, holding them there for a number of hours and then cooling slowly to room temperature. No optimum time-temperature schedule was discovered and so it suffices to state that the entire procedure took a day and a half or more.

Heat-treated crystals were not perfectly annealed but their strains had so largely disappeared that they were generally suitable in most unpolarized applications. A majority of them exhibited no colour patterns when examined between crossed polarizers. Interferometric tests made on a more or less typical specimen, in the form of an optical flat, indicated clearly that the maximum variation in path length for green light was less than  $1/8$  wave length per inch of thickness.\*

**Artificial Fluorite Properties.**—Needless to say, not all of the crystal castings were optically perfect because some were grown from unknown stock for test purposes and others had not had the benefit of complete scavenging. A great majority of those grown from first-class fluorspar were colourless.

\* The flat and measurements were made at Mt. Wilson Observatory and reported by Dr. T. Dunham, Jr.

Seven colourless crystal specimens were reported to transmit ultra violet to 1160-1310 Å through thicknesses of several millimetres; one, to only 1390 Å.\* No others were tested. One colourless crystal specimen, 31 mm. thick, was found to transmit infra red to 9.8  $\mu$ . It was the only one tested.

Refractive indices of three crystals grown from different fluorspar under different conditions are given in Table I.

TABLE I

Spar Origin	Scavenger	$n_D$ for 15° C	Laboratory
Arizona .. ..	No	1.43389	Eastman Kodak Co., Rochester, N.Y.
New Mexico .. ..	Yes	1.433898	{ National Bureau of Standards, Washington, D.C.
Kentucky .. ..	Yes	1.433888	

All of the data are in sufficiently close agreement with accepted values to indicate that there is very little inherent difference between artificial and natural optical fluorite. The major difference is in size. Whereas the natural material is nearly always small and is relatively scarce, large artificial crystal castings can now be manufactured in great quantities.

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\* The measurements were made in the Physical Measurements Laboratory, M.I.T.

## IMPROVED CRYSTALLIZATION OF LITHIUM FLUORIDE OF OPTICAL QUALITY

BY DONALD C. STOCKBARGER

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Optical lithium fluoride is not a new material. It was being made on a small scale, although in practical sizes, in academic laboratories more than ten years ago. Moreover, its methods and means of production were described,<sup>1,2,3</sup> and would seem therefore to require no further treatment were it not for the facts that (a) certain original unpublished details appear to be of greater importance than they did earlier, and (b) striking improvements have followed in the wake of the wartime research on artificial fluorite. This paper is intended to bring the subject of lithium fluoride production up to date.

Refractive indices and spectral transmissions are of prime importance in an optical crystal. They are usually influenced more by chemical composition than by physical structure, in the case of an isotropic material, and the transmission characteristics are especially sensitive. The topics

<sup>1</sup> Ramsperger and Melvin, *J. Opt. Soc. Amer.*, and *R.S.I.*, 1927, **15**, 359.

<sup>2</sup> Schneider, *Physic. Rev.*, 1936, **49**, 341.

<sup>3</sup> Stockbarger, *R.S.I.*, 1936, **7**, 133.

requiring the closest attention are therefore the establishment and the maintenance of chemical purity of exceptionally high order.

Of the many possible ways of synthesizing LiF a favourite one consists in mixing aqueous  $\text{LiHCO}_3$  and HF solutions. First of all, the  $\text{LiHCO}_3$  is prepared by bubbling  $\text{CO}_2$  through saturated aqueous  $\text{Li}_2\text{CO}_3$  solution in a clean vessel at room temperature. It is helpful to use sufficient excess  $\text{Li}_2\text{CO}_3$  to give a nearly saturated solution of  $\text{LiHCO}_3$  and the conversion is facilitated by agitation and causing the  $\text{CO}_2$  pressure within the vessel to build up to a few pounds per square inch. Obviously, only the best  $\text{Li}_2\text{CO}_3$  is suitable and so it may be important to prepare it by a special chemical procedure.

The  $\text{LiHCO}_3$  solution is filtered repeatedly until clear and free from sediment and then is mixed with purified HF solution in some manner ensuring neutralization in acid environment. The latter specification is emphasized because inclusion of basic molecules in the salt needs to be avoided. An excellent procedure is to add the  $\text{LiHCO}_3$  to the HF slowly with agitation, stopping while acid is still present in excess. The LiF precipitate may be washed with dilute HF solution, or sparingly with water, and drained by decantation, for example. In any case, it is still acid when placed in a ventilated oven whose temperature is not much above that of the room, say,  $40^\circ\text{C}$ , where it dries quickly if spread out thinly.

The selection of reaction vessels, stirrers, etc., is always guided more or less by availability and so cannot be specified rigidly. It must, however, be made with due regard to chemical neutrality and cleanliness lest new impurities be introduced. In fact, the entire synthesis procedure and the subsequent handling of the LiF are performed with this thought in mind. Dust of all kinds, for example, is studiously avoided; clean garments are worn and smoking is strictly prohibited in the vicinity.

The crystallization process itself is brought about by "slow" cooling of the melt, preferably in a manner assuring the continuous growth of a single lattice structure. The well-known accompanying tendency to exclude foreign particles, such as ions of different materials, is enhanced by maintenance of a flow of heat across the liquid-solid interface whose magnitude greatly exceeds the rate of latent-heat liberation. The exclusion tendency—purification—is also affected by slow freezing, but not necessarily favourably as will be shown.

The excess heat flow from melt to solid is a natural consequence of the temperature gradient commonly employed to facilitate ordered growth. It evidently brings about the rejection of impurities, accidentally lodged in the interface solid layer, through the associated vigorous bombardment which depends on the departure from Maxwellian energy distribution. Its effectiveness may be expected to be related to the first, second and even higher derivatives of temperature with respect to distance across the interface region, for the bombs come from the melt layer and their penetration is a function of the solid layer plasticity. This view is amply supported by the results of numerous critical experiments.

With high-grade salt, largely uncontaminated by residual deleterious impurities and protected from the entrance of external ones, the principal remaining problems lie in the reduction of accidental pollution. There is strong evidence gathered throughout the growth of lithium fluoride crystals, from synthesis to crystallization, that the most likely accident is reaction with water.<sup>2</sup> This is commonly termed "hydrolysis," but it may well be more far reaching in its nature and consequences in this instance than is the simple process suggested by examples and discussions found in treatises on chemistry.

It evidently can make its appearance during the drying of the salt (compare behaviour of calcium fluoride<sup>4</sup>) and can become prominent during the sintering and melting. It continues during crystallization in room air so that there is a conflict between contamination and purification analogous to an ordinary reversible chemical reaction tending to proceed in opposite directions simultaneously.

In the hypothetical absence of continual contamination and of natural or accidental thermal disturbances, very slow crystallization of a fairly good melt might be expected to result in some excellent optical material, even without high excess heat flow, because presumably there would be sufficient time for wandering impurities to diffuse out of the way of the

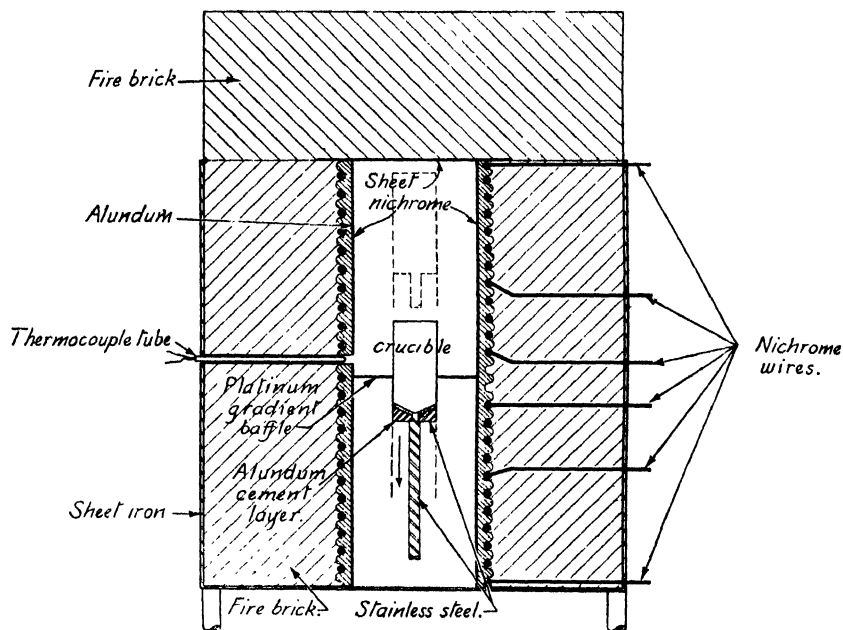


FIG. 1.

advancing solid surface. In the presence of accompanying hydrolytic contamination, however, it is often observed that increasing the length of crystallizing time, beyond a fairly well-defined value dependent on thermal conditions, fails to bring about noticeable improvement in quality. Therefore, in contrast with purification data obtained under quite different circumstances,<sup>5</sup> the temperature gradient can become paramount in importance.

The considerations of the preceding paragraph can account in part for the frequent observation that crystal quality is not uniform along the direction of growth. Some such inhomogeneity may be found, however, even in the absence of continual melt contamination because the initial melt impurities, rejected by the growing crystal, increase in concentration with time to the extent that they decrease the effectiveness of the purification process.

A preferred means for crystallizing alkali halides in air<sup>3</sup> is indicated diagrammatically in Fig. 1. This differs from the more familiar apparatus

<sup>4</sup> O.S.R.D. Report, No. 4690.

<sup>5</sup> McFee, *J. Chem. Physics*, 1947, **15**, 856.

introduced by Bridgman<sup>6</sup> in that it possesses novel features, particularly helpful in the case of lithium fluoride, which are described below.

The salt is contained in a thin-walled platinum crucible whose conically shaped bottom is held on a metal seat by a layer of Alundum cement cast in place. The seat is supported by a metal elevator rod leading to a mechanism designed to lower the assembly at any one of many speeds as low as 0.1 mm. per hour. The rod, turned to a diameter of a few millimetres at the upper end, projects through the cement-coated seat to cool the crucible apex selectively during the initial freezing.

A polished platinum annular baffle-plate provides thermal shielding between the upper (hotter) and lower wire-wound furnaces so that the crucible passes through a nonlinear gradient whose maximum value is relatively high. In practice, the baffle is often a sandwich of Nichrome between two thin sheets of platinum. Stainless steel can be substituted for the Nichrome here as well as in other places although it corrodes faster. The clearance between the crucible and baffle window is nearly uniform around the circumference and may be about 1 mm. The refractory furnace cores are usually lined with sheet Nichrome to lengthen their lives by limiting the access of LiF vapour.

Opposite the thermocouple tube there is a small horizontal cylindrical passage (not shown), through the outer casing into the interior of the furnace, whose axis lies in the annular baffle. This permits occasional insertion of a platinum alloy wire, attached to the end of a Nichrome rod 2 or 3 mm. in diameter, to determine the location of the freezing level by probing the soft crucible wall very gently. The passage must be kept filled with a suitable plug between probe measurements to prevent distortion of the thermal field.

The provision for probing has been of great usefulness in controlling the crystal-melt interface position. The optimum level for purification, in furnaces similar to the one sketched, has been found to be a little below the baffle in agreement with theory. Optional features include a heated cover in place of the insulating brick on top and a closure at the bottom of the furnace.

Accurate means for controlling both temperature<sup>7</sup> and line voltage<sup>4</sup> are generally necessary since fluctuation of the freezing level may be dangerous. It needs to be remembered, however, that means for excluding the causes of fluctuation can be far superior to means for their regulation, especially if the latter tend to hunt. For example, in the complete absence of the objectionable influences of air currents and ambient temperature changes, it may be better to dispense with all attempts to regulate the furnace temperature, but only if the line voltage is held practically constant. There is no established limit to the desired precision of control. Usually the best devices are none too good and commercial instruments are likely to be unsatisfactory. The matter is of such great importance that it has become and remains the subject of a group of research programmes.

For use in the presence of air the crucible may advantageously be made of pure platinum approximately 0.003 in. thick. It can be fabricated successfully in the laboratory through spinning and hammer-welding, or it may now be purchased from manufacturers of noble metal articles. The material should be free from foreign particles often carelessly embedded during the rolling of the sheets. The exterior of the vessel is lightly copper-plated and the interior is cleaned chemically before use. The copper becomes oxidized and so improves the transfer of radiant heat.

<sup>6</sup> Bridgman, *Proc. Amer. Acad.*, 1925, **60**, 305.

<sup>7</sup> Stockbarger, *R.S.I.*, 1939, **10**, 205.

When crystallization characterized by high purification has been permitted to proceed to completion in air, corrosive impurities rejected by the LiF are deposited over the upper part of the crucible wall where they sometimes do serious damage. The best known method of protection is to halt the controlled crystallization prematurely so that the impurities are largely entrapped in the top layer of the frozen mass. With this possible exception the platinum ordinarily exhibits no sign of attack.

The crucible is emptied through superficial melting, as taught originally by Slater<sup>8</sup> and later by others,<sup>9,10</sup> the method being similar to that once commonly employed to release ice cubes in the domestic kitchen. It can be patched with pieces of 0.001 in. sheet platinum if necessary, restored to shape and dimensions by stretching and spinning over a suitable form and then re-used after cleaning and replating.

The manner of introducing the LiF into the crucible in air has influence on the quality of the final product, presumably on account of its effect in limiting hydrolysis. Two practical methods have been adopted. The first consists in (a) sintering the more or less fluffy powder quickly in a heavy-walled platinum crucible to form dense buttons, (b) crushing the sintered masses to pass a  $\frac{1}{4}$ -in. mesh screen, (c) loading cold and covering the vessel with a suitable platinum lid, and (d) fusing the salt in the crystallizing furnace rapidly. If step (d) is prolonged the granules may turn brown.

The second method consists in (a) preparing a shallow layer of melt as above, and (b) introducing the unsintered precipitate, little by little, with the crucible kept covered between partial loadings. Care is exercised to keep the melt well above the fusion point and each stage of the loading is performed quickly with the lid removed only briefly.

Many lithium fluoride crystals were grown in air at M.I.T. prior to the entrance of the U.S.A. into the war. They differed in ultra-violet transmission characteristics as did those produced in other academic laboratories.<sup>2,11</sup> They also exhibited the defect of colour which ranged from practically none through light yellow to brown approaching opacity and some of them scattered light noticeably.

It is remarkable that the fusion procedure, like the preparation and the drying of the salt, can have influence on the colour of the crystal. To add emphasis it may well be stated here that melting point samples, repeatedly fused and frozen in the open, have been observed to turn deeply coloured. Moreover, partial melting back of a crystal followed by solidification in the normal manner has left a clearly defined layer of strongly coloured debris within the final product.

Critical experiments designed to correlate ultra-violet and visible defects with known variables demonstrated clearly the necessity of meticulous care and control from beginning to end. In doing so they also proved that the apparatus and procedures described above could produce useful material. For example, crystals known to have favourable historical backgrounds were found to transmit radiation of wavelengths less than  $1100 \text{ \AA}^*$  in agreement with published data on selected crystals grown elsewhere.<sup>2,11</sup>

Although unsuspected at the time, at least a majority of the crystals undoubtedly possessed the defect of selective infra-red absorption which

<sup>8</sup> Slater, *Proc. Amer. Acad.*, 1926, **61**, 135.

<sup>9</sup> Strong, *Physic. Rev.*, 1930, **36**, 1663.

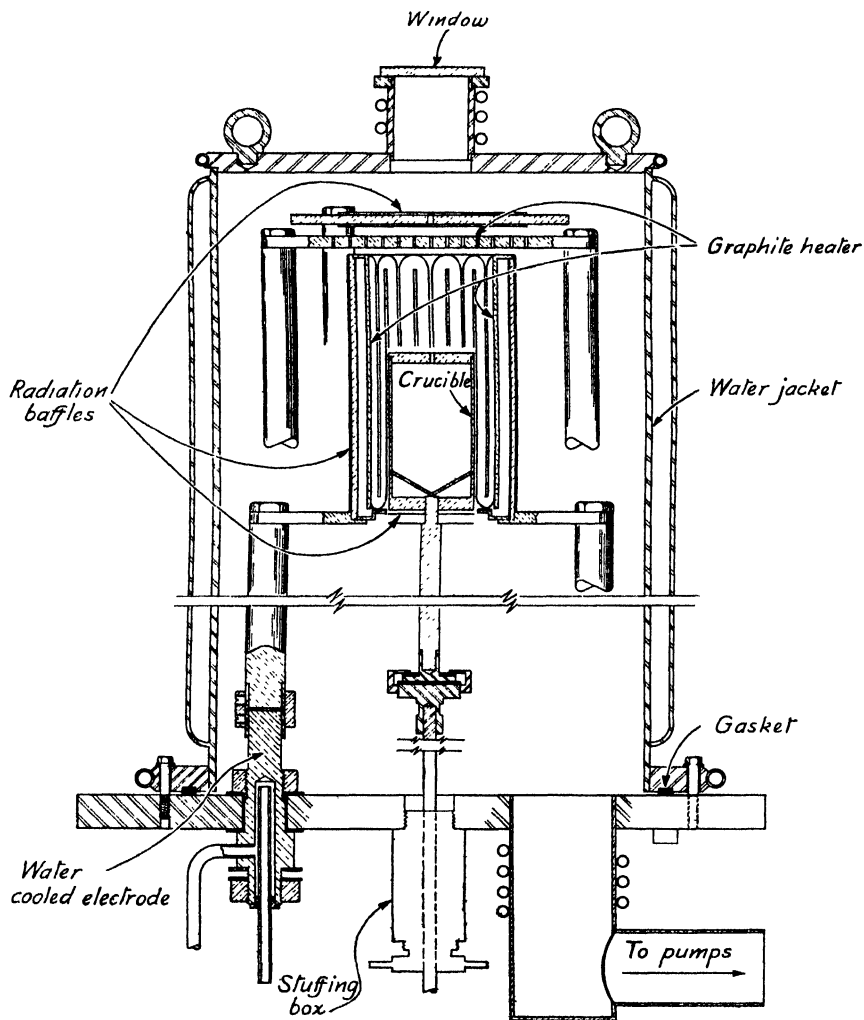
<sup>10</sup> Stockbarger, *J. Opt. Soc. Amer.*, 1937, **2**, 416.

<sup>11</sup> Melvin, *Physic. Rev.*, 1931, **37**, 1230.

\* The measurements were made by Mr. H. W. Allen in the M.I.T. Spectroscopic Laboratory.



has been found more recently in commercial air-grown lithium fluoride.<sup>12</sup> Since most of the commercial material is somewhat coloured and since colourless lithium fluoride, grown during the wartime research, has been found to be free from this defect, it is a temptation to associate the selective infra-red absorption with visible colour. Actually, any relation between the two has not been established directly.



"VACUUM" FURNACE.

FIG. 2.

It may well be added here that no relationship between unnatural ultra-violet absorption and either visible colour or selective infra-red absorption by lithium fluoride has been proven conclusively.

A special "vacuum" type of crystallization furnace<sup>4</sup> developed for growing artificial fluorite during the war can be used profitably for lithium fluoride.

<sup>12</sup> Wright, R.S.I., 1944, 15, 22.

It is shown schematically in Fig. 2. Fundamentally it differs little from the atmospheric furnace and so requires only brief description. Its heating elements, either spirals or grids, are made of graphite, and all hot metal parts of molybdenum. A magnetically operated tungsten wire probe (not shown in the figure) is lowered through the melt to determine the freezing level.\*

The entire housing is water-cooled and is so well constructed that the internal gas pressure can be maintained at a small fraction of a micron throughout normal use. Consequently this furnace possesses the advantages of (a) shielding from external thermal disturbances so that no temperature regulator is required, and (b) practical elimination of chemical reactions between the salt and the atmosphere.

A crucible which was developed particularly for fluoride growth, but which evidently is generally useful when out of contact with air, is very easily turned on the lathe from a graphite rod. The machinist merely bores and reams a hole and then turns the exterior with the support of an appropriate, carefully centred mandrel. The process is much simpler than the metal working involved in making a suitable platinum crucible. The only real difficulties are encountered in the selection of the graphite which must be dense, flawless and free from impurities which cannot be removed readily. The crucible is cleaned by leaching with molten salt ( $\text{LiF}$  here), most simply accomplished by maintaining a load well above the melting point for one or more days. It can be emptied by inversion at room temperature because the frozen salt mass does not adhere to the graphite.

All graphite is more or less porous even in the absence of flaws and consequently the wall thickness can seldom be reduced below 0.03 in. without danger of serious leakage. The fraction of satisfactory crucibles made in this manner may not be large for this as well as other suggested reasons but the expenditure of effort is justified by the enjoyment of graphite's practical advantages.

Only a few vacuum-grown lithium fluoride crystals were produced during the war. They were made in a single furnace of obsolete design, having no probe, from fragments of a rather deeply coloured air-grown crystal known to possess poor ultra-violet transmission. All of the specimens were colourless. One crystal, approximately 25 mm. thick after polishing, was tested. It transmitted infra-red to about  $6.5\ \mu$  with no evidence of selective absorption, proving that the previously noted infra-red defect was not an intrinsic characteristic of the substance  $\text{LiF}$ . It was opaque in the ultra-violet below  $0.2\ \mu$ , showing that whatever purification there may have been during the freezing had been insufficient to convert an inferior melt into a wholly satisfactory crystal. Much better ultra-violet-transmitting, colourless crystals were grown in later experiments with a less obsolete furnace and different  $\text{LiF}$  stock. A few specimen plates were transparent below  $1100\ \text{\AA}$ .

Unquestionably the new type of furnace is better than the atmospheric one in several respects, but it is also much more expensive and difficult to construct and operate. Although it readily produces colourless lithium fluoride crystals, especially suitable for infra-red work, it manifestly does not eliminate the necessity of meticulous care in the preparation of salt intended for ultra-violet-transmitting material. Consequently it probably will not replace the older type for average, small-scale crystal growing. \*

Some of the apparatus details and operating procedures presented herein may seem to be needlessly meticulous. Quite possibly they could be

\* An illustrated description of the apparatus and methods for growing artificial fluoride will appear in *J. Opt. Soc. Amer.*

replaced by others, but long experience has demonstrated their superiority over many well-tried alternatives and recognition of their value has grown increasingly with understanding of their roles. Their descriptions are necessarily incomplete and are intended primarily as guides toward the growth of lithium fluoride crystals suitable for optical use.

Two physical characteristics of lithium fluoride which have been known for over ten years are presented here. The second one in particular is being studied further at the time of this writing.

The first has to do with parting. Some lithium fluoride crystals crack cleanly along a 110 plane about as readily as they split in the 100 direction usually associated with normal cleavage. Consequently it is sometimes difficult to determine orientation through the customary preliminary breaking test and, moreover, plate fragments may possess triangular and other strange outlines.

The second is concerned with plasticity. It has been found that lithium fluoride plates can be bent dry and at ordinary temperatures to radii of  $\frac{1}{2}$  in. or less. Thickness, up to a few millimetres at least, appears to be unimportant and danger of failure is small provided that the physical structure is good.

Much of the equipment used in the prewar research was purchased with funds granted for the purpose by the Rumford Committee of the American Academy of Arts and Sciences. The apparatus used during the war was the property of the National Defence Research Committee, U.S.A.

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## THE GROWING OF CRYSTALS

by the methods of Kyropoulos and of Stöber

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Large single crystals are required from which to fashion optical components, particularly for infra-red apparatus, for lenses and for polarizers. They are also wanted for experiments in crystal physics and chemistry and for electrical purposes especially as piezoelectric oscillators. Our interest in them was first aroused through requiring crystals for our experiments in light scattering in the alkali halides (second-order Raman effect).\*

We considered the various processes and decided that the one likely to serve us best was that of Kyropoulos, in which the crystal is drawn from the melt. We chose this method, since we were likely to need a number of different alkali halides, and it had the special advantage that one could see what one was doing, so that for a new substance one would quickly find the correct conditions for growth.

\* Much useful information is contained in some of the reports of B.I.O.S., notably in No. 468 (Grove-White), I.G., Ludwigshafen, Oppau, No. 552 (Coates), Göttingen and Copenhagen, and No. 1579 (Menzies, Skinner & Rands), Göttingen, I.G., Munich.

There were substances, however, such as sodium nitrate, which seemed less suited to the Kyropoulos process, so we decided to try also the method of Stöber, especially since to the best of our knowledge no one else in this country was using that method. In it, heating and cooling are applied to the material in such a way that isothermal planes move up through the material, which is at rest. In some ways it can be regarded as a converse of the Bridgeman method.

In this paper we give a brief account of the two methods, the results achieved with them by various workers, with different substances, and the advantages and limitations of the two processes.

### The Kyropoulos Process

The process has been described by Kyropoulos,<sup>1,2</sup> and much work on it has been carried out in the First Physical Institute in Göttingen under the direction of Prof. R. Pohl, so that it is sometimes connected also with his name. Krüger<sup>3</sup> in Denmark has also worked by this method.

It is convenient to divide the description into two main parts: (1) growing and (2) cooling. The process will first be briefly outlined, and then special details will be mentioned.

#### Growing the Crystal

A seed crystal is held in a chuck made from pure nickel, forming the lower end of a vertical water-cooled nickel tube, and is supported over the mouth of a vertical furnace, inside which is the crucible containing the salt. The furnace temperature is raised, the salt melts and the temperature is raised still further and maintained at 10°–20° above the melting point, to get rid of all traces of crystallinity. The seed is then lowered so that the lower end enters the liquid, and it is allowed partially to melt, so as to form a fresh surface. The temperature is then made to fall, so that crystallization on the seed commences. By some (e.g., Göttingen school) this is accomplished by turning on the cooling water at this point, a drop at a time to begin with, but we prefer to keep the water flowing all the time, even at the beginning, and bring about the initial cooling by lowering the furnace temperature.

The crystal grows downwards and outwards (particularly the latter), the new growth forming a bun-shaped addition to the seed. If left to itself, it would grow out until it reached the side of the vessel. This is prevented by raising the chuck and seed, so that the lower surface of the bun is still just in the liquid. Another bun is then added to the crystal, and so the process continues. As crystallization proceeds, the furnace heating is progressively reduced. Finally the crystal is broken off the end of the seed, and cooled down.

**Cooling the Crystal.**—The crystal may be cooled in the same furnace in which it has been grown; by lifting the crystal just clear of the residual melt, one may allow the furnace to commence cooling, and when the melt has frozen gently let the crystal down on to the top of it, when the seed will normally snap off and the chuck may be withdrawn. Alternatively a second furnace of a simple kind may be ready, at a temperature just below the melting point, to receive the crystal. We have used both methods, and the chuck supports are so designed that the crystal may be swung round into a second furnace.

The temperature of the cooling furnace is then reduced. Opinion among different workers varies concerning the safe rate of cooling. In the Göttingen school, the temperature is reduced rapidly at first, and then more slowly. We have so far found little difference between the results of switching off the furnace and allowing it to cool at its own rate, or of arranging slower controlled rates of cooling. These remarks apply to NaCl and KBr. The rate should not be so rapid that the crystal cracks, nor that there is an undue amount of strain introduced. The cooling is a matter for further study.

<sup>1</sup> Kyropoulos, *Z. anorg. Chem.*, 1926, **154**, 308.

<sup>2</sup> Kyropoulos, *Z. Physik*, 1930, **63**, 849.

<sup>3</sup> Krüger, *Fysisk. Tids.*, Copenhagen, 1942, **40**, 17.

**Crucibles.**—For the vessel which is to hold the melt, all that is required is something which will not crack too easily on heating, which will stand up to the temperature to be employed and which does not react with or dissolve in the melt itself. Pyrex glass is suitable for silver chloride (m.p.  $455^{\circ}\text{C}$ ); porcelain (glazed or unglazed) is satisfactory for most of the alkali halides with the exception of the fluorides which attack it. For the fluorides a platinum vessel is essential. For some salts silica vessels have been used.

**Metal Chuck.**—The chuck which supports the seed crystal should be made of a good thermal conductor; it must not oxidize rapidly at the temperatures reached and it should not react with the crystal itself. Nickel is suitable provided its temperature is kept fairly low by a small flow of water, but if the water is turned completely off it oxidizes rapidly. It must also be kept free from condensed water vapour otherwise it is corroded by sublimed salt which settles on to it. Water condensation on the cool upper parts of the chuck can be a considerable nuisance, since it tends to run down into the furnace and causes flakes of salt, which have sublimed on to the cold metal, to fall off on to the top of the crystal or into the melt. These flakes are invariably coloured green due to reaction with the nickel and so contaminate the melt. The water condensation can be avoided by running the cooling water at such a speed that the temperature of the outer surface of the pipe is above the dew-point.

**Seed Crystal.**—A suitable crystal to be used as a seed must be obtained: once the process is successfully in operation, this is not difficult. We find a small furnace and gear useful for growing seeds. It is not essential to use the same material as a seed at the commencement: we have found it possible to start growing  $\text{NaNO}_3$  by using a calcite seed.

The size of the seed used depends upon the intended size of the finished crystal, for the seed has to carry the weight. For rocksalt crystals about 5 in. across, we find a seed of 1 in. diam. suitable, while for crystals 9 in. across, we use seed of 2 in. diam.

### Control of Rate of Growth and Limit of Crystal Size

The rate of growth of the crystal can be controlled either by the degree of cooling applied through the chuck, or by varying the temperature of the melt. It is usually necessary to vary both of these factors; initially the rate of water flow is kept small and the melt temperature may be  $30^{\circ}$  or  $40^{\circ}\text{C}$  above the freezing point. As the crystal gets larger the water flow has less effect and the final control is achieved by varying the temperature of the melt. At any particular temperature of the melt and degree of cooling applied, the crystal will grow until an equilibrium state is reached where the heat supplied to the melt by the furnace in a given time is equal to the heat conducted away by the cooling system. When this stage has been reached the crystal can only be forced to increase in size by reducing the input to the furnace which results in lowering the melt temperature (assuming that water cooling is now a maximum). Eventually the temperature of the melt is only slightly above the freezing point and the temperature gradient across the growing surface of the crystal becomes very small. At this stage spontaneous crystallization is likely to occur and the rate of growth can no longer be controlled. This situation determines the practical limit to which a given combination of furnace and cooling system will operate successfully. It limits the size to which a crystal can be grown. In order to grow crystals of greater depth, a water-cooled spiral which can be lowered on to the top of the crystal to supplement the cooling of the chuck is helpful. For sodium chloride and potassium bromide a spiral of copper tube well plated with nickel is suitable. The inlet and outlet tubes should be supported in such a manner that they may be manipulated so that the contact between the spiral and the top surface of the crystal is as good as possible.

**Uneven Growth.**—If the furnace is not evenly lagged all round, or its temperature is not symmetrical about the vertical axis, then the growing crystal will tend to grow more rapidly on the side facing the coolest part of the furnace wall. This causes uneven growth and reduces the ultimate size of crystal which is obtained, since it must be lifted when the resulting bulge approaches the side of the crucible and this will occur before the rest of the crystal has

reached its full diameter. This trouble is difficult to eliminate completely, and perhaps the easiest way is to arrange for a slow rotation of the crystal as it grows. A symmetrical crystal then results (Körber).

**The Lifting of the Crystal.**—The speed with which the crystal is lifted depends upon a number of factors. As crystallization takes place a diminution of volume of the melt occurs. This fact in itself causes the level of the melt to fall away from the crystal, and if the diameter of the crystal is not much smaller than that of the crucible from which it is being grown there is no need to lift the crystal at all. However, if the crystal is much smaller in diameter than the vessel containing the melt, the liquid level is not much affected by the growing crystal, and the latter must be lifted from time to time; rates of lifting of about 2 to 4 mm./hr. are usually satisfactory. The rate of growth should be controlled so that a rate of lifting of this order is maintained.

### Crystals Grown by the Kyropoulos Process

For the most part, crystals grown by this process have been alkali halides, or mixtures of them.

**Single Substances.**—We have grown crystals of the following materials:

- (a) in large sizes (i.e., over 4 in. diam. and 3 in. deep)—NaCl, KBr;
- (b) in smaller sizes—NaBr, RbBr, AgCl.

In none of these was any particular difficulty encountered. AgCl, contrary to general belief, presents no particular difficulty apart from having to work in artificial light. In addition to the above, others have reported growing KCl, KI in large size, and in small sizes LiF, NaF, RbCl, RbI, CsCl, TiCl, TiBr, AgBr.

**Mixed Materials.**—Many mixed substances have been investigated at Göttingen, where it has been found, for example, that crystals of KCl + KBr, and RbCl + RbBr can be grown in mixtures of any proportions, and in general that this is true of any mixture of two salts provided that the crystal class is the same and that the radii of the two anions are not greatly different, and likewise those of the two cations. The crystal grown will not, in general, be constant in composition down the cylinder. Dr. A. Hammer while at Steinheil's, Munich, studied a number of mixtures: those successfully grown were KCl + KBr, BaF<sub>2</sub> + SrF<sub>2</sub> and TiCl + TiBr ("K.R.S. 6").

**Very Large Crystals.**—As a matter of interest, the largest crystal we have seen made by this process is one obtained by Dr. Körber, of I.G. Farbenindustrie, Ludwigshafen, Oppau; it was a cylinder of rocksalt about 12 in. across and 6½ in. high, and of good quality.

### Advantages and Limitations

With this method of crystal growth the initial stage consists of growing on a chosen seed crystal suspended in the chuck. Since the seed itself is a single crystal there is every likelihood that the resulting crystal will be single: furthermore its axes will be orientated in the same way as those of the original seed. The orientation of the crystal axes relative to the shape of the lump of crystal obtained is therefore determined by the way in which the seed crystal is cut. Crystals like sodium nitrate, however, will only grow satisfactorily when the axis of greatest thermal conductivity is arranged to be along the direction of heat flow. Thus there is only one way of mounting the seed crystal—that is, with this axis vertical.

The process can be interrupted during growth. If, for example, too rapid a growth has been allowed to occur, resulting in a cloudy crystal, the melt temperature can be raised and the faulty portion of the crystal melted away. The temperature is then slowly lowered again so that the correct rate of growth is obtained. Of course, it is not possible during growth to see whether the crystal is cloudy or not, but if too rapid a growth is taking place at the start numerous radial lines appear on the top surface when the crystal is increasing in diameter.

When the crystal is fully grown there is no difficulty in breaking it away from the top of the seed which still remains held in the chuck. The crystal itself is thus free as it is cooling, and is not subjected to any strain due to the contraction of any containing vessel.

As mentioned earlier, there is a limit to the thickness of crystal which can be grown by a given furnace and cooling system. There comes a time when the rate at which heat is being conducted away from the top of the crystal cannot be made to exceed the rate at which heat is being supplied to it by the furnace, unless the melt temperature is allowed to fall too near to the freezing point. When this happens spontaneous crystallization takes place and the resulting growth is neither controllable nor single.

Unless many crystals have been grown under identical conditions, the control of the rate of growth and the size of the crystal depend upon visual observation. It is necessary therefore to open the top of the furnace from time to time, or to have a permanent peep-hole. Great care must be exercised in examining the state of the growth in this manner or irregular growth results. A good illuminating system which can be directed into the furnace is a great help.

### Stöber Process

A method of growing crystals from the melt which has been used with considerable success to grow large single crystals of sodium nitrate has been described by Stöber.<sup>4</sup>

A vessel, roughly hemispherical in shape, containing the material to be crystallized is placed inside a well-lagged chamber and heated from above until its temperature is somewhat higher than the melting point of the substance, and held there until its contents are molten. The temperature of the lowest point of the vessel is then slowly reduced by applying cooling from below while the temperature at the top of the melt is kept constant. If the apparatus is well designed the isothermal surfaces are flat, and as the cooling from below becomes more vigorous the whole system of isothermals moves upwards. The position of the isothermal surface corresponding to the temperature of solidification determines at any time the location of the solid-liquid boundary. Crystallization begins when this isothermal surface reaches the lowest point of the vessel and then proceeds upwards through the melt. In this way crystallization begins at a point and a single crystal results. It is necessary in the final stages to lower the temperature at the top of the melt.

Stöber used the method to grow crystals of sodium nitrate of 3-4 kg. He used a pure nickel vessel cooled by means of a water-fed spiral below the melt. The experiment lasted 8 days but was held stationary each night. He pointed out that for the method to succeed the material must be able to withstand temperatures somewhat above its melting point, and that the degree of overheating necessary is dependent upon the viscosity of the melt when only just above the freezing point. Sodium nitrate, for instance, would grow quite well with only a small temperature gradient, but bismuth required a much greater one.

The method has to be modified when the molten material behaves like water (below 4° C), where the density is decreasing as the temperature falls to the freezing point. When this occurs the cooling from below sets up large convection currents in the liquid, which disturb the process of crystallization. It can be overcome by cooling from above so that the temperature gradient is reversed.

Like all methods in which crystallization takes place under the influence of a temperature gradient, the resulting crystal (unless it is isotropic) is orientated so that its axis of greatest thermal conductivity lies along the lines of heat flow. For this reason it is important that the isothermal surfaces should be as nearly plane and parallel to each other as possible otherwise a single crystal will not be obtained. Stöber used the method with success for materials of melting points up to that of NaCl, but pointed out that with suitably designed apparatus the method is general and could be used for substances with much higher melting points.

Almost the same method is being used to grow crystals of sodium nitrate by G. Weissenberg in the University of Marburg. Weissenberg places the  $\text{NaNO}_3$  in a thin Pyrex vessel, the shape of an evaporating basin, which is then put into a well-lagged chamber between an upper and a lower heater. After the material has been brought to the liquid state he arranges a constant difference of temperature between the top and bottom of the vessel and slowly lowers

<sup>4</sup> Stöber, *Z. Krist.*, 1925, **61**, 299.

both together. He obtains single crystals the size of the vessel (about 7 cm. diam. at the top). The same method has been set up in the research laboratories of Hilger & Watts Limited and a number of attempts have been made to grow  $\text{NaNO}_3$  crystals, but only partial success has so far been achieved.

### Growing of Organic Crystals

A modified form of Stöber's method has been used with success for growing crystals of organic materials, as, for example, *p*-dichlorobenzene and naphthalene, grown by Rousset and Lochet.<sup>5</sup>

The material is melted in a large crystallizing dish, 15 cm. diam., and the depth of liquid is about 2 cm. It is kept hot by the heat radiated to it from an electric heater of large surface, several centimetres above the dish. The heater is energized from a constant voltage source, and initially is sufficiently hot to keep the liquid molten.

The current through the heater is slowly diminished to zero over a period of about 2 days by the device of including a liquid resistance in series, and allowing this liquid to drip away steadily. The isothermal planes consequently slowly rise through the liquid, and solidification takes place steadily. The solid mass which results usually contains four or five single crystals of 10 to 20 cm.<sup>3</sup> volume.

### Limitations of the Stöber Method

Since the temperature gradient has to be maintained throughout the whole depth of the melt this places a limit on the thickness of the crystals of any one substance which can be grown by this process. The temperature of the highest point of the melt will be limited by the boiling point of the liquid or possibly by a value at which decomposition occurs. The temperature of the lowest point of the melt will, at the beginning of crystallization, be at the freezing point. For a particular substance there will be a minimum temperature gradient, which must be exceeded if satisfactory crystals are to be grown. These three factors clearly place a limiting value on the linear distance between the highest and lowest points of the melt and therefore on the thickness of the finished crystal.

The finished crystal has to be cooled down to room temperature in the same vessel in which it has grown; for this reason the vessel should be thin and thus enable the cooling crystal to alter its shape as it contracts. Too strong a vessel will cause the crystal to fracture on cooling owing to the internal strain set up.

### Test for Uniqueness of a Cubic Crystal

Prof. Mollwo, in Prof. Pohl's laboratory, showed us a useful test to decide whether an uncut crystal is or is not single. The mass is held in the hand so that light is reflected into the eye from the cylindrical surface. The cylinder is rotated about its axis and, if the crystal is single, it will be found that the reflected light waxes and wanes four times in a revolution. The brightest reflections occur when the eye is directed towards a part of the cylinder surface approximately parallel to the cube surface of the seed crystal, and the duller when the light is being scattered off the "corners" of the cubes which make up the crystal. With practice, one can follow these places of maximum brightness down the length of the crystal, and observe if they lie on directions straight and parallel to the axis. The test applies in the above form, when (as is usual) the seed has one cubic axis vertical.

**Appendix.** Since writing this paper, some interesting facts have come to light concerning work in this country on infra-red materials, for which we are indebted to Prof. R. V. Jones of Aberdeen.

Windows of rolled silver chloride, and one of silver bromide, were made for Sir James Dewar by Hilgers in 1919, and they were mentioned by him in a discourse given to the Royal Institution in January, 1920. It seems probable that the discovery of the interesting transmission of silver chloride in the infra-red is to be ascribed to Dewar.

<sup>5</sup> Rousset and Lochet, *J. Physique*, 1942, 3, 146.



Crystals of silver chloride and silver bromide of about one half-pound in weight were grown by Jones in Oxford in 1937, using the Kyropoulos method. Crystals of the alkali halides have been grown by him recently, using a stainless-steel cooler. He finds that a crystal of an alkali halide grown from the melt appears to cleave as a single crystal, but close examination of a cleaved surface reveals a number of spindle-shaped sub-crystals which are not exactly orientated alike, but have the long directions of the spindles approximately along the direction of growth. He is attempting to improve the singleness of crystals grown from seeds, by fitting the seed in the chuck with its previous direction of growth horizontal, so that the surface projecting into the melt, on which the growth will take place, exposes the spindles sideways instead of end-on.

He has grown crystals of sodium nitrate and naphthalene, and is planning to grow the fluorides of lithium and calcium.

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## GROWING SINGLE CRYSTALS FROM SOLUTION

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Both because of the rich diversity of phenomena attending crystal growth, and because those who grow crystals usually do so to obtain the crystals rather than to study the process, crystal growing remains more an art than a science. The apparatus and procedures described here have formed over some six years a fairly satisfactory basis for the practice of the art in our own and a few other laboratories, when modified to accord with the aforementioned diversity, and it may be hoped that they might provide a tool for investigating some aspects of crystal growth more exactly. They have been applied most widely to the laboratory production of ionic crystals from aqueous solution for studies of their elastic, dielectric, and piezoelectric properties.

When, as customarily, the solubility of the salt to be crystallized increases with temperature, it is more satisfactory, because more readily controllable, to grow crystals by slowly cooling the solution than by evaporating it. What rate of cooling is suitable varies, of course, with the ratio of growing area to volume of solution, the temperature coefficient of solubility, and the tolerable unflawed growth rate of the material. Rough rules to guide a preliminary experiment are (1) salts with solubilities in the range 20 to 50 weight-% (or about 5 to 10 mole-%) will tolerate a linear advance of the fastest growing faces of 0.05" to 0.10" per day (or about 100 to 200 molecular layers per second), and (2) the lower the solubility, the lower the tolerable rate.

"Tolerable growth rate" means the rate above which "veils"—threadlike inclusions of mother liquor—begin to be included in the growing crystal. Veils appear usually to be consequences of inequalities of supersaturation of the mother liquor over various areas of a single face, arising from differences in the readiness of replacement by fresh solution of exhausted solution next to the growing face, differences which increase as the face gets larger. Hence the mother liquor must be forcibly circulated over the growing faces in order to obtain practical growth rates on large crystals.

Here enters the limitation that crystal growing deals, for several weeks continuously, with a thermodynamically unstable system, tending to deposit crystal nuclei spontaneously, the more readily the more it is agitated. Any circulating system should, therefore, be one in which the maximum disturbance occurs where it will do some good, at the crystal surfaces, and this is most simply achieved by moving the crystals through the otherwise unagitated solution. The whole system should be mechanically and hydrodynamically as simple as possible, avoiding cracks and ledges and "dead" spots where spurious crystals can become inconvenient. At best it should afford some means of destroying the spurious crystals.

The "reciprocating rotary crystallizer," embodying these considerations, has been most successful as a laboratory tool\* in the following form. A cylindrical jar of Pyrex glass, a foot in diameter and a foot-and-a-half high, acts as container for about 24 l. of mother liquor and as its own thermostat. It sits on an enclosed annular air space about an inch deep containing unsheathed Nichrome-coil heating elements. One set of coils, below the centre of the jar, continuously dissipates 30 W. Another coil, beneath the periphery of the jar, dissipates 100 W under control of a heavy-duty relay and a thermal regulator of the sealed-contact mercury-in-glass type.

The lid of the jar (of stainless steel, or of cold-rolled steel with  $\frac{1}{8}$ " sheet rubber cemented to the under side to retard rusting) is drilled in the centre to clear a shaft which carries and moves the crystals. Commonly the shaft is of 1" polymethyl methacrylate rod, 15" long, turned down to  $\frac{1}{2}$ " for an inch projecting through the lid, and the crystals are supported on spoke-like cross-arms of  $\frac{1}{8}$ " stainless steel rod, carried in tightly fitting holes drilled in the shaft, and projecting about 3" from it. The lid is also drilled to take rubber stoppers, one carrying a thermometer, and another the thermal regulator, if it is adjustable, or a test-tube in which a fixed thermal regulator will loosely fit so that it can be removed for resetting without exposing the solution to dust from outside, and in which a little water or oil improves thermal contact between regulator and solution.

The crystals are carried through the solution at the ends of the cross-arms by rotating the central rod at rates of from 15 to 30 rev./min., depending on the viscosity of the solution and its stability to supersaturation. If spurious crystals are formed, they find their way to the bottom of the jar and are carried to the centre of the bottom by the vortical motion of the solution. That section of the bottom is slightly warmer than the bulk of the solution, because of the continuous heater beneath it, and the spurious crystals are either dissolved or much slowed in growth.

Unless the direction of rotation of the shaft is reversed at least once a minute, the solution rotates with the crystals and relative motion declines, and the crystals show veils on the "wake" side, evidencing dead spots. Midget motors, reversed by relays controlled by clock motors through cams operating micro-switches, afford elegant driving units of the required type. Simpler, and adapted to driving several crystallizing units, is belting from pulleys on a jack shaft driven as follows. A continuous motor turns, through reducing gear, a crank at the rate of about 4 rev./min., from which a crank shaft drives a large bicycle sprocket, rotating it about one-third revolution forwards and back. From this sprocket a roller chain drives a small sprocket on the jack shaft, one-third revolution of the former providing three revolutions of the latter.

\* Pilot-plant adaptations for 300-l. capacity by Dr. A. C. Walker, and manufacturing equipment designed by the Western Electric Company with Dr. Walker's assistance, will not be described here. Both are designed for growth at constant temperature and continuous resaturation at a higher temperature.

Commonly it is satisfactory to approximate the desired continuously declining temperature by setting fixed regulators in decrements of  $0.2^{\circ}\text{C}$ , and sometimes decrements as large as  $0.5^{\circ}\text{C}$  are tolerable. The temperature "hunting" fluctuation never exceeds  $\pm 0.05^{\circ}\text{C}$ . In ordinary laboratory ambients, the apparatus is conveniently operable up to about  $50^{\circ}\text{C}$ . At temperatures above  $40^{\circ}\text{C}$  a jacket of  $\frac{1}{4}$ " felt may be placed around the jar if the ambient is subject to large fluctuations; the jacket does not extend above the level of the liquid, since it is advisable that condensation continually take place on the walls, washing them down. At temperatures above about  $55^{\circ}\text{C}$  complications enter from evaporation, crusting at a meniscus, and the like, unless the shaft enters through a liquid seal and all other joints are tight.

The most suitable seeding technique varies with size and substance. In initial work, when only small crystals are available, it is suitable to force over the supporting spoke a snugly fitting length of plastic tubing, of the type used for electrical insulation, with about  $\frac{1}{8}$ " projecting from the end of the spoke, and then to force a small seed crystal into the hollow section so that it is firmly held but projects slightly. When larger seeds are available, they may be drilled with two blind holes,  $\frac{1}{16}$ " diam. and  $\frac{1}{4}$ " apart for example, and two correspondingly spaced spokes may be used to support the crystals by first inserting the tubing into the holes and then forcing the spokes into the tubing. Crystals can usually be drilled with ordinary twist drills rotated at high speed and applied with very small pressure, and with a strong air blast directed down the drill. The use of the tubing provides a snug resilient mount; but, more importantly, makes it possible to harvest the crystals without breaking them, by first withdrawing the spokes and then permitting the tubing to collapse and slide out of the holes.

In general, any fragment of crystal is a possible seed, but commonly one showing only natural faces is best. In many cases of materials which grow in prisms terminated by pyramids, however, plates with their major surfaces normal to the prism axis make excellent seeds. In the initial stages of growth the plate becomes "capped" relatively rapidly with pyramids which grow fastest from the edges of the plate, and which when completed enclose a pyramidal volume of mother liquor and thereafter advance cleanly. This technique becomes of importance when growth rates are so much higher along than across the prism axis that the length outruns the equipment before the girth is sufficient for the intended use. Successive operations using successively larger plates may then avail.

In planting the seeds, they must be brought to the planting temperature in an air thermostat so that they will not crack, as can conveniently be done by placing the seed-spoke-rod-lid assembly in a Pyrex glass cylinder, like the jars but bottomless, placed over one of the heater-relay units. Planting must always be done in unsaturated solutions, to dissolve dust of the material inevitably introduced. Suitable technique is to warm the solution to about  $2^{\circ}\text{C}$  above the saturation temperature, quickly to transfer the seed assembly from the air thermostat to the jar and start it turning, and to cool the solution to the growing temperature, about  $0.2^{\circ}\text{C}$  below saturation, over one to three hours, depending on the size of the seeds and the velocity of dissolution: a time sufficient to ensure solution of spurious solid and insufficient to loosen the seeds from their mounts. In harvesting the crystals it is safest to syphon out the solution, leaving the crystals to cool slowly in the moist, warm, felt-wrapped jar overnight.

Clearly dust and chips of the material to be crystallized, or of materials isomorphous with it, must be avoided, but it is less clear that miscellaneous dust forms crystallization nuclei in a supersaturated solution. In practice,

however, such dust may wipe and abrade nuclei from a growing crystal, and hence is kept to the ordinary level of chemical operations, as for example by suction filtration through a "filter aid" in a Buchner funnel.

When the scale of the apparatus is reduced to 4-l. capacity, using jars 6" diam. and a foot high, it becomes of especial importance to design the heater base so that it has low heat capacity, in order to minimize temperature fluctuations. The higher meniscus-to-volume ratio makes it necessary also to take greater precaution at all temperatures against evaporation leaks.

The principles on which the apparatus described is based have been successfully embodied in a modification of the 4-l. apparatus for growing crystals of materials whose solubilities decrease with increasing temperature. The jar stands on three short legs in a shallow metal pan of water, with a constant-level attachment, so that the bottom of the jar is immersed to a depth of about  $\frac{1}{2}$ " and is thus cooled. The heat input is distributed around a central zone by a fine Nichrome-wire winding on the jar, covered on the outside with felt. The crystals are grown in the zone immediately above the heated zone, and the temperature is, of course, progressively raised.

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## THE GROWTH OF LARGE CRYSTALS OF AMMONIUM DIHYDROGEN PHOSPHATE AND LITHIUM SULPHATE

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The work discussed in this paper is concerned with the growing of large single crystals of ammonium dihydrogen phosphate (ADP) and lithium sulphate. Both of these materials are water-soluble and the methods employed for their growth are based upon deposition from saturated solution.

The crystal as finally grown in its final form has to furnish a number of plate elements for piezoelectric purposes, and these elements, in addition to being cut in specific direction in relation to the crystal axis, must be of certain minimum dimensions. To be acceptable therefore the crystal should not only be large; it should also be well-proportioned.

**MODIFICATION OF CRYSTAL HABITS.** To secure these well-proportioned crystals often calls for special measures of adjustment and control of the growing operations. Crystals growing under completely unregulated conditions from a solution of the "pure" substance are apt to show preferential growth along one axis, and measures must therefore be devised to modify this tendency. It has been found, for example, that in the case of the long tetragonal crystals of ADP there is necessity in the first stage to encourage early growth along the  $x$ -axis and this can be effected by increasing the pH of the solution by addition of the di-ammonium salt. In the case of lithium sulphate the flat monoclinic crystals require, in the first stage, to be encouraged to make increased growth along both  $x$ - and  $z$ -axes. Here the means employed is to lower the pH of the solution by addition of  $H_2SO_4$ . In both cases the required girth of the crystal once established can be maintained during succeeding development by a correct choice of conditions.

### Ammonium Dihydrogen Phosphate Crystals

**General Outlines of the Process.** ADP crystals are grown by immersing crystal seeds in a saturated solution of the material and then cooling the solution in a carefully controlled manner with some stirring. The seeds are flat plates cut from a fully grown crystal. The growth is conveniently carried out in two stages. The first stage (known as "capping") develops the natural pyramidal ends on the flat plates. This growth is attained by cooling a saturated solution from 35°–28° C over a period of 8 to 10 days. The second stage (known as "bar growing") continues the growth in the lengthwise direction and requires a cooling period of 4 to 5 weeks during which the saturated solution is cooled from 50° C to room temperature. The normal ADP bar has a total length of 8 to 10 in. and a square section approximately of 1½ in. edge. This cross-section is of optimum dimensions for the present requirements. When crystals of this cross-section are available for seed cutting, further growth on the prism faces is inhibited by the addition of traces of iron to the growing solution.

**Process Details.** The crystal-growing process consists in immersing seeds in a saturated solution which is then slowly cooled and it may be conveniently studied by considering the stages in detail.

(a) **SEEDS.** Any slices cut normal to the  $z$ -axis of a crystal may be used as seeds provided the faces normal to the  $x$ -axis are intact; the end pyramids and slices cut almost up to the original seed may be used. A cut slice is about ¼ in. thick and in it holes are drilled to take two stainless steel pins to support the seed in a vertical position on the frame which fits the bottom of the growing tank.

#### (b) SOLUTION PREPARATION

(i) "*Capping*" Solution. About 110 l. of solution saturated at 35° C approximately are prepared by dissolving 47 kg. of commercial ammonium dihydrogen phosphate (for typical analysis see footnote (a)) in 95 l. of distilled water, conductivity about 9 megohm.

(ii) *Bar Growing Solution.* About 110 l. (or double this quantity if the double tank unit is used) of solution saturated at 50° C approximately are prepared by dissolving 62 kg. of ADP in 90 l. of water. To this solution, however, iron is added as inhibitor in the proportion of 0.06 g./l. (see footnote (b)). The approximate saturation temperature is checked by determining the specific gravity of the solution. These solutions are prepared in a 400 l. "dissolving" tank, which carries a steam heating coil and high speed stirrer constructed throughout in F.D.P. stainless steel. Its capacity is sufficient for 2 full charges, with ample room for stirring.

(iii) *Filtering.* Filtration is carried out by means of a Metafilter, Type P.S.2, size 2, through a prepared bed of Metasil A. The solution is pumped through the filter and returned to the dissolving tank until the filtrate is quite clear and bright when examined by a strong light beam. The clear filtrate is pumped into the intermediate tank. In this tank the solution is slowly cooled to the saturation temperature as found by specific gravity, and thereafter the precise saturation temperature is determined by testing with a small seed; erosion of the seed edges or evidence of growth on the faces will be seen after immersion of the seed for 4 hr. in a solution which is over 0.05° C above or below the saturation point. The solution temperature is then raised 0.5° C above saturation point to allow for cooling during transfer and also to dissolve any crystal debris on the seeds when delivered to the growing tank.

(a) **Typical Analysis:**—ADP, clean white crystals; volatile matter (100° C) 0.01 %; insoluble in water 0.01 %; chloride (as Cl<sup>-</sup>) 0.002 %; sulphate (as SO<sub>4</sub><sup>2-</sup>) < 0.0005 %; nitrate (as NO<sub>3</sub><sup>-</sup>) < 0.001 %; iron (as Fe) 0.0018 %; other metals < 0.01 %; pH 1.0 M solution, 4.1.

(b) The optimum cross-section of crystal bars for making units is about 1½" and once this has been attained growth along the  $z$ -axis only is required. The presence of Fe, Al or Cr tends to inhibit cross-sectional growth, and the amount added is somewhat critical within ± 25 %; too little will allow the bar to fatten; too much will cause it to taper until growth almost ceases.

(iv) "*Capping*." The capping unit consists of a stainless steel tank  $4' \times 2' \times 1'$  deep enclosed in a mild steel tank. A heating element is mounted between the bottoms of the 2 tanks and this is controlled by a resistance and a motor-driven contact thermometer for a thermostat fitted in the solution tank. A removable carrier constructed of Perspex strips on a stainless steel frame covers the bottom of the solution tank. This frame mounts 76 seeds. The whole unit is mounted in bearings in a rigid frame, and rocking motion imparted to it by means of a 1 H.P. motor through a Croft's 140 to 1 reduction gear, producing 11 complete rocking cycles per minute with a movement through an arc of  $20^\circ$  (i.e.  $10^\circ$  each side of the horizontal). The seeds mounted on the frame are slowly heated (for about 24 hr.) to the solution temperature, and when this is correctly adjusted the solution is run in from the intermediate tank.

The solution is maintained slightly above saturation point for 24 hr. to clear completely any undissolved salt. Then the cooling programme operates starting with a reduction in temperature of  $0.25^\circ \text{C}$  over the first 24 hr. followed by a 24-hr. period of  $0.50^\circ \text{C}$  and thereafter the maximum cooling of  $1.0^\circ \text{C}$  per day is carried on. In all, a cooling range of about  $7^\circ \text{C}$  is allowed (from  $35^\circ$  to  $28^\circ \text{C}$  taking about 8 days) and is designed to allow the pyramidal ends to be developed and then enclosed completely in  $\frac{1}{8}$  in. layer of clear growth.

(v) *Bar Growing*. Bar growing is carried out in a 2-tier rocking tank unit which, with its driving mechanism, is housed in a "hot" room. The 2 stainless steel tanks and their mechanical operation is similar to the capping unit, only the method of controlling the temperature differs, in this case the surroundings of the unit, i.e., the whole room is temperature-controlled. The room is constructed so that one end is closed by means of heavy insulated doors which give full access to the room for loading and unloading tanks and entrance or removal of any gear. When the room is in operation these doors are permanently closed, and access to the room is by means of an air lock. The room is lit by fluorescent tube lights and the double windows in one wall permit observation at any time. All the controls are brought to the outside of the room and there are automatic recorders of both air and solution temperature.

The procedure is exactly the same as that for capping, with the substitution of "capped" seeds for seeds and the extension of the cooling range to about  $25^\circ \text{C}$  (i.e., from  $50^\circ \text{C}$  to normal).

### Lithium Sulphate Crystals

**General Outlines of the Process.** The monohydrate  $\text{Li}_2\text{SO}_4 \cdot \text{H}_2\text{O}$  has a small negative temperature coefficient of solubility which precludes the method of growing crystals by slowly cooling (or heating) the saturated solution. The method used is that of maintaining the solution at a fixed temperature and permitting a controlled rate of evaporation of water from the solution. The development of the process has not yet reached full scale and is at present being carried out in large-scale laboratory ware.

**SEEDS.** Slices are cut parallel to the main growing faces ( $Q$  faces) about  $\frac{1}{4}$  in thick and they are drilled for mounting on stainless steel wires.

**SOLUTION PREPARATION.** Commercial salt is purified by recrystallization from boiling solution acidified with sulphuric acid. After recrystallization the salt is dissolved in cold water and filtered. Before use the pH is adjusted (usually to pH 6) and its saturation point adjusted by reference to its specific gravity.

**CRYSTAL GROWING.** The seeds are mounted on stainless steel wires (sleeved with polythene tubing) which form radial arms of a crystal holder. The holder is gripped in a chuck which is carried in bearings and fitted with a drive which can be rotated with a reversing motion. The rotation is about 25 rev./min., the direction being reversed every second revolution.

The growing vessel is a 35-l. Pyrex vessel with cover. The general arrangement of the vessel with its heating jacket is shown in Fig. 1a. The manner in which water is withdrawn from the system is shown in Fig. 1b. The cover acts as an air-cooled reflux condenser and the condensed vapour is collected in a copper water collector. Water is withdrawn from this collector to correspond with growing rates known from experience. The excess condensed water overflows the rim of the collector and falls on to the surface of the solution and so forms a



thin layer of cooled, unsaturated solution which tends to prevent surface crystallization which is apt to be so troublesome in this work. The surrounding water jacket is maintained at constant temperature by means of an immersion heater and Sunvic controller. The growing temperature is 80°–85° C.

The vessel is filled with cold solution and the mounted seeds immersed. Assembly of the unit is completed and the temperature of the vessel raised as quickly as possible by means of the heating jacket, taking about 7 to 8 hr. This period at unsaturated condition completely clears the system of undissolved salt arising from crystal debris. At the end of the growing period, which is about four weeks for a 3- to 4-in. crystal, the solution is syphoned out and the vessel slowly cooled (at a rate of 1° to 2° C per hour) by means of a motor-operated Sunvic controller.

**CRYSTAL HABIT.** The habit of the lithium sulphate crystal is considerably affected by the pH of the growing solutions. To obtain crystals of suitable girth, growth at pH 4 to 5 is necessary. When the necessary girth is obtained, lengthwise growth is encouraged by higher pHs, say 6 to 7.

**General Comments on Growing Crystals.** **TEMPERATURE CONTROL.** It will be appreciated that temperature control over considerable periods (4 to 5 weeks) is critical, and failure is one of the main causes of flaws in crystals.

**FLAWS IN CRYSTAL GROWTH.** (i) **AMMONIUM DIHYDROGEN PHOSPHATE.** The only flaws experienced in ADP crystals are veils (thin layers of disordered growth) parallel to the pyramid faces. These can arise, of course, by failure of the temperature control but there is a possibility of their infrequent occurrence when the temperature control is not suspect. Possible causes are unequal circulation of solution around growing crystals and chance deposition or adsorption of impurities on the advancing faces. External disturbances, e.g., vibration, cannot be overlooked.

(ii) **LITHIUM SULPHATE.** Three types of flaws have been experienced in lithium sulphate crystals: (a) the crystal is optically imperfect throughout. Crystals of this type show much faster growth (3 times that of the sound crystal); (b) growth is considerably or even completely inhibited at one polar end of a crystal; (c) cracks in the crystal, usually along cleavage planes, but often rather irregular. The cracks may occur during growth, but cases occur when an apparently sound crystal cracks during storage 1–15 days after growing and cooling.

All these effects are considered to be due, in some degree, to adsorption of impurities originally present in the growing solution. Some work on this problem is in progress. Some evidence has been accumulated to show that flaw type (c) is due to the growth of one habit being superimposed on another habit. E.g., change of pH is known to change habit; seeds grown at one pH and then grown in solution of another pH frequently show this type of cracking.

*Royal Naval Scientific Service.*

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## CONTROLLED GROWTH INHIBITION IN LARGE-SCALE CRYSTAL GROWTH

BY HANS JAFFE AND BENGT R. F. KJELLGREN

*Received 21st February, 1949*

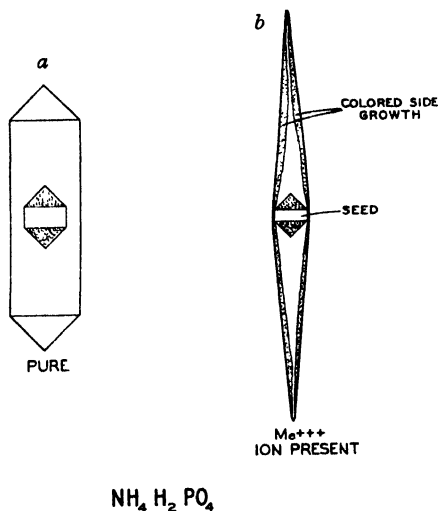
The piezoelectric properties of ammonium dihydrogen phosphate (ADP) as recognized about 1941 made these crystals highly suitable material for certain types of electro-mechanical transducers. We were faced with the problem of producing these crystals in quantities of thousands of pounds within a few months after conclusion of our laboratory-scale experiments. One phase of this work was based on the realization that impurities first



regarded as a severe impediment for crystal growth were beneficial if properly controlled.<sup>1</sup> The present paper deals with this occurrence.

Crystals of ammonium dihydrogen phosphate, as well as the isomorphous phosphates and arsenates, almost invariably show a very simple habit, the combination of a tetragonal prism and tetragonal bipyramid. Notable variations occur, however, in the relative development of these two forms and hence in the shape of the crystals which may vary from stubby to acicular. In the latter type, a tapering of the prism faces is frequently noted; the pyramid faces may thus be entirely suppressed. It had been noted that the stubby habit is favoured in less acid solutions.<sup>2</sup>

When the project of ADP crystal growth was transferred from the laboratory to plant scale, we soon found that the tendency of crystals to grow in elongated shape and to develop tapering prism faces became more pronounced as the solutions aged. This effect was readily traced to contamination by iron from the pumping system used in transferring the crystallizing solutions. Similar but more extreme development of tapering with almost complete



suppression of the prism faces was noted in an experiment where a small amount of chromium was added to an ADP solution. Fig. 1 *a* and 1 *b* show, respectively, a crystal grown from a pure solution and a crystal grown from a solution containing chromium. Similar crystal seeds and analogous temperature conditions were chosen for both crystals. The chromium had been added in the form of the bichromate ion and was present primarily as such in the solution at the end of the run. However, there was a beautiful deep green coloration of the crystal, indicated by shading in Fig. 1 *b*, due to trivalent chromium obtained by reduction caused by some organic cements in the crystal-growing tray. Study of the discoloured crystal shows that the green colour is essentially limited to those parts of the crystal which grew by deposition on the prism faces. The sharp boundary between the coloured and uncoloured part of the crystal is the locus of the successive positions of the edges between prism and pyramid. Frequently it is observed that the colour is more pronounced along this locus than farther out in the material

<sup>1</sup> Kjellgren and Jaffe, *U.S. Pat.* 2,452,576 (1948).

<sup>2</sup> Busch, *Helv. phys. Acta*, 1938, **11**, 273.

deposited on the prism faces. This indicates that the trivalent chromium ion and similarly the trivalent ferric ion is adsorbed preferentially at the edges between the prism and pyramid faces. It was later found that the trivalent aluminium ion has the same effect. The influence of the three ions declines in the series chromium-iron-aluminium. We are inclined to believe that most of the known influence of acidity on habit of ADP and possibly potassium dihydrogen phosphate is due to the varying activity of these trivalent ions as a function of acidity. If the pH exceeds about 4.5 these metals will be precipitated as their phosphates.

When iron was recognized as the factor responsible for the gradual change of growing conditions in our solutions at the crystal plant, steps were taken to eliminate this contamination, but soon unexpected difficulties arose. The practice of crystal growth involved the cutting of seed plates perpendicular to the optic axis having a cross-section almost as large as the desired crystal. When we switched to solutions thoroughly freed of iron, so much lateral growth of the crystals occurred that their length development was less than desired; variability of shape was a hindrance for cutting operations. One of us (B.R.F.K.) therefore introduced the practice of maintaining a known small concentration of iron such as 0.1 g./l. in order to have a predictable growth character. It was soon found that this addition not only produced the desired limitation of lateral growth, but also permitted a much faster growth on the pyramid faces than could previously be obtained. Further experiments with all three named trivalent ions showed that this increase of growing rate on the pyramid faces was due to higher oversaturations maintainable in the crystallizing solutions when these ions had been added. Addition of iron in the amount mentioned raises the rate of undercooling that can be maintained in ADP solutions without excessive demands on exclusion of dust, etc., from around one or two degrees centigrade to about twice that value. One centigrade undercooling corresponds to 7.5 g./l. excess ADP salt. More extreme conditions prevail with potassium dihydrogen phosphate solution containing chromium where we could maintain an oversaturation corresponding to a differential in saturation temperature of 12° C in the presence of a crystal seed. With this combination of potassium dihydrogen phosphate and chromium ion, inhibition unfortunately affects growth on the pyramid faces as well as the prism faces and is therefore of doubtful value.

The following picture of the cause underlying this increased range of oversaturation is proposed: any microscopic crystal nucleus forming in the solution will at once act as a centre of attraction for the trivalent ions present which will block growth of the nucleus in directions perpendicular to the optic axis. The nucleus thus prevented from expanding in cross-section will be subject to dissolution by the statistical fluctuations in the solution unless the oversaturation is increased well above the limit of stability for pure solutions. On the basis of this picture we can expand the principle of increased crystallization speed by selective growth inhibition to other crystal types and other growth inhibitors. In order to benefit from growth inhibitors, it is necessary that there be sets of faces on the crystal which are subject to different growth inhibiting action by the chosen inhibitor. The set of most strongly inhibited faces must satisfy certain conditions. On the one hand, it must not be a closed crystallographic form or closed combination of forms, as such a set of faces would soon surround the whole growing crystal and inhibit all growth, as mentioned with potassium dihydrogen phosphate and chromium. Thus, the method is not applicable to cubic crystals because all forms in the cubic system are closed. On the other hand, the set of inhibited faces must be sufficient to prevent growth of a nucleus into a three-dimensionally extended body. Inhibition on only a positive pyramid in a crystal

of one of the pyramidal (polar) symmetries would, for instance, not be sufficient.

The minimum useful set consists of two parallel faces, such as a pinacoid ; this will restrict growth to two dimensions. A practical example of this case is lithium sulphate monohydrate which is known to grow freely in all directions from an acid solution but shows tabular habit if grown from a neutral or alkaline solution. We have verified that the oversaturation obtainable in the neutral and alkaline solutions of lithium sulphate is higher than in acid solutions. The other principal useful case is inhibition on a set of faces forming a closed zone, permitting unrestricted growth in one dimension only, as exemplified by ADP with iron.

In order to make practical use of the increased speed of growth in selected directions by adding inhibitors for growth in other directions it is necessary, of course, to provide a seed which has satisfactory dimensions in those directions along which further extension of the crystal is limited by the inhibitor. Large-scale growth of such crystals therefore divides itself into two steps. The first is the provision of satisfactory seed crystal material grown in the absence of the inhibitor. The second is the growth of large crystals in the inhibited solution from plates or blocks cut from the seed crystal such as to show large extension in the directions of inhibition.

No molecular picture for the adsorption of the trivalent ions of chromium, iron and aluminium on the primary phosphate crystals has been formed. It is suggested that this adsorption is related to the low solubility of the phosphates of these metals even in moderately acid solution.

There are two other ions whose presence in ammonium dihydrogen phosphate solutions has an important effect on the properties of the crystals grown.<sup>3</sup> These are the bivalent barium and sulphate ions. The former produces some growth inhibition on the prism faces, but the outstanding effect of both ions is a tremendous increase of the electric conductivity of the crystal. At 25° C, the resistivity of an ADP crystal grown from the purest solution we could obtain (about 1 part sulphate ion per 100,000 parts phosphate ion) is near 30,000 megohms cm. ; a solution of a typical grade of technical ammonium dihydrogen phosphate containing one part sulphate per 1000 parts phosphate is 2000 megohms cm. ; and the resistivity of a crystal grown from a solution containing 5 % sulphate is 100 megohms cm. The same increase in conductivity is produced by about half the corresponding molar amounts of barium. The actual concentration of sulphate ion in the crystal is found to be one-tenth of that in the crystal growing solution if it is near two parts sulphate per 10,000 parts phosphate in the latter. The ratio, (sulphate in crystal)/(sulphate in solution), decreases with increasing sulphate concentration.

As precipitation of barium sulphate from ADP solutions is incomplete special methods were needed for sulphate analysis. These were developed by The Bell Telephone Laboratories where also detailed studies of ADP crystal resistivity against temperature were made.<sup>4</sup>

The free entry of the sulphate and barium ions into the ADP lattice is explained by agreement of their radii with those of the  $\text{PO}_4^{---}$  and  $\text{NH}_4^+$  groups respectively. The differences in valency must be compensated by a deficiency of hydrogen ions. The conductivity introduced by these impurities may be interpreted as "proton hole" conduction.

*The Brush Development Company,  
Cleveland 14,  
Ohio.*

<sup>3</sup> Jaffe, *U.S. Pat.* 2,449,484 (1948).

<sup>4</sup> Murphy, *Physic. Rev.*, 1945, **68**, 1283.

# HYDROTHERMAL SYNTHESIS OF MINERALS

BY JEAN WYART

*Received 10th March, 1949*

Some mineralogists and petrographers think that water plays an essential role in the genesis of minerals and rocks. It is this role which I am trying to determine by making hydrothermal syntheses.

## Experimental

Two types of autoclaves are used here :—

(i) Small ones (internal volume 20 cm.<sup>3</sup>, 15 cm. long and 4 cm. diam.) made of special steel, which can withstand a pressure of 700 kg./cm.<sup>2</sup> at 500° C. They are closed with a Bridgman joint. Five of such autoclaves can be placed in the same electrical oven, thus allowing a more rapid study of the influence of one parameter, e.g., the proportion of a component in the initial mixture, at a known temperature. Unfortunately the pressure cannot be measured directly ; it is calculated from a knowledge of the quantity of water added at the beginning of the experiment and of the free volume of the vapour.

(ii) A bigger autoclave (internal volume 500 cm.<sup>3</sup>) which withstands a pressure of 400 kg./cm. at 500° C. The pressure is measured on a manometer but the temperature is not known with accuracy because the head of the autoclave is outside the oven.

Usually, a silver laboratory tube of internal volume 100 cm.<sup>3</sup> is used with this autoclave. It is hung in the autoclave and heated by the water vapour. From the cover of the silver tube a crucible is suspended. The action of the liquid and that of the vapour can therefore be studied separately.

**The Function of the "Supercritical" Water Vapour.** Vitreous silica powder is placed in the silver tube with pure water, sufficient in quantity for the vapour to remain saturated up to the critical temperature.

Silica does not crystallize if the temperature is less than 374° ( $\pm 5^\circ$ ) C, which is the critical temperature. Above this temperature, the vitreous silica changes to cristobalite. The latter does not undergo any transformation under the action of the pure water vapour alone at 500° for eight days. In the same conditions tridymite crystallizes to quartz. It seems that, at this temperature, cristobalite is more stable than tridymite.

Let us suppose that instead of pure water a dilute potash solution (1/100 mole/l.) is used. The silica is placed in the crucible at the top of the silver tube so that it is submitted only to the action of the vapour. As long as the temperature is below 370°, the amorphous silica does not crystallize as in the previous experiment. But if the temperature exceeds the critical temperature, the water vapour rapidly transforms the silica first to cristobalite, and then to quartz. It is evident that there is a dissolution of potash in the "supercritical vapour" and a change in the chemical properties of the water vapour above the critical point.

This vapour can dissolve metals so that the synthetic product can be considerably influenced by the walls of the tube. Thus, in an experiment using slightly "potassified" water vapour at 490° under 580 kg./cm.<sup>2</sup>, beautiful copper octahedrons were found. The silver tube in fact contained a little copper which by diffusion was dissolved in the vapour.

**Mechanism of the Crystallization of Silica.** Micrographs show clearly that the crystallization of the silica glass begins at the surface and continues progressively into the interior. One can imagine that the molecules of silica, as they dissolve in the vapour, give to the neighbouring atoms of the solid an energy sufficient to enable them to reach a more stable equilibrium state, corresponding to cristobalite if the solvent power of the vapour is weak, or corresponding to quartz if it is stronger (i.e., with "potassified" water vapour).

**Principal Syntheses of Minerals.** Repeating previous experiments of C. and G. Friedel who submitted white mica to a potash solution at  $500^\circ$ , crystals of orthoclase  $\text{KAlSi}_3\text{O}_8$  were obtained. These were about 1 mm. long and were recognizable by their shape, their optical properties and their X-ray patterns. At the same time there were produced hexagonal crystals in the form of hexagonal lamellæ (00.1) and short prisms (10.0) which C. and G. Friedel identified as nepheline, but which, in reality, were kalsilite  $\text{KAlSiO}_4$ .

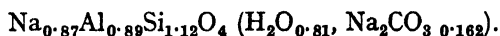
The  $\text{K}_2\text{O}-\text{Al}_2\text{O}_3-\text{SiO}_2$  system was studied in the presence of "supercritical" water at temperatures ranging from  $380^\circ$  to  $500^\circ$  C. The initial products were amorphous; the amorphous silica and alumina were pulverized and were mixed and the potash was dissolved in the water. In this way fine crystals were rarely obtained, even in prolonged experiments (8 days); the final products can be identified by their X-ray patterns.

By using a mixture of  $\text{Al}_2\text{O}_3 + 2\text{SiO}_2$ , one can very easily obtain kalsilite  $\text{KAlSiO}_4$ . With an excess of silica, this gives orthoclase  $\text{KAlSi}_3\text{O}_8$  immediately; and in spite of numerous attempts, the intermediary product which is leucite  $\text{KAlSi}_2\text{O}_6$  was never reproduced. When a potash solution reacts on amorphous alumina and an excess of silica, one obtains finally a mixture of orthoclase and quartz.

**Study of the System  $\text{Na}_x\text{K}_{1-x}\text{AlSiO}_4$ .** With Miss Mireille Michel-Lévy, the author has been studying the products resulting from a mixture of amorphous  $\text{K}_2\text{O}$ ,  $\text{Na}_2\text{O}$ ,  $\text{Al}_2\text{O}_3$ ,  $\text{SiO}_2$  in the stoichiometric proportions corresponding to  $\text{Na}_x\text{K}_{1-x}\text{AlSiO}_4$ . The experiments were made in the small autoclaves, the products being placed in copper tubes. When  $x$  is less than 0.55, they obtained kalsilite from  $360^\circ$ . When  $x$  lies between 0.55 and 0.80 at  $380^\circ$  nepheline appeared in hexagonal prisms, reaching 1/10 mm. in length, while at  $360^\circ$  they obtained cancrinite but not in a well-crystallized form.

If we use pure soda, the nepheline does not crystallize at first. At temperatures of  $360^\circ$  to  $420^\circ$ , cancrinite is formed, whose parameters are:  $a = 12.65 \pm 0.02$  Å,  $c = 5.15 \pm 0.02$  Å, with a small quantity of analcite. At  $480^\circ$  cubic crystals of the sodalite family appear with  $a = 9.02$  Å.

If instead of stoichiometric proportions we use soda in excess, cancrinite and sodalite separates as well-developed crystals, the first in hexagonal prisms with the indices:  $\epsilon_D = 1.489$ ,  $\omega_D = 1.492$ ; the second with the forms (100) (110) and the index  $n_D = 1.478$ . Chemical analysis of the cancrinite corresponds to the formula:



Finally when one submits the cancrinite previously obtained to the action of a solution rich in soda at  $500^\circ$ , Na-nepheline is formed as fine crystalline hexagonal prisms of length 0.5 mm.



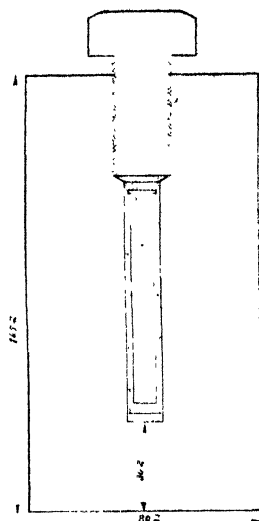


FIG. 1. Diagram of the steel bomb used for the initial explosion and subsequent annealing that produced artificial silicate minerals.

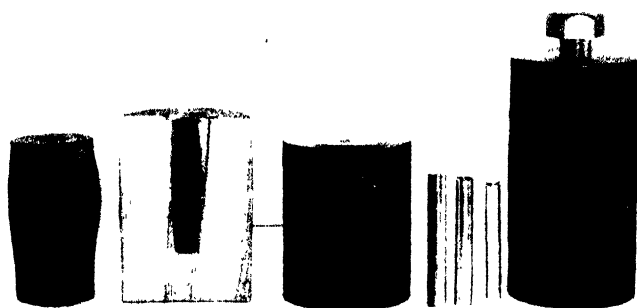


FIG. 2. A steel bomb (cf. Fig. 1) before and after explosion and annealing

# THE SYNTHESIS OF CRYSTALS PRODUCED IN GASEOUS MEDIA BY DETONATION OF EXPLOSIVE MIXTURES

By A. MICHEL-LÉVY

*Received 31st January, 1949*

It is of interest to recall how fruitful experiments on crystal synthesis of minerals produced in gaseous media by mixtures of finely ground products and explosives have been. Ing. Ge. H. Muraour and I have shown that a finely crystallized explosive such as Hexogene produces in argon by virtue of the shock waves an intense luminous phenomenon of short duration (about  $4 \times 10^{-6}$  sec.) and pressures of about 50,000 kg./cm.<sup>2</sup> and temperatures of 30,000° C. The composition of the gases obtained from the Hexogene is: CO<sub>2</sub>, 3.97 %; CO, 9.53 %; H<sub>2</sub>O, 9.53 %; H<sub>2</sub>, 3.97 %; N<sub>2</sub>, 13.5 %. When  $\frac{1}{8}$  to  $\frac{1}{4}$  by weight of finely ground powders of various constituents (e.g., silica glass, alumina, alkaline salts, etc.; minerals, such as quartz, feldspars, pyroxene, olivine, etc.; sugar carbon, diamond dust, powdered rocks, granite, kimberlite, etc.) are intimately mixed with the Hexogene, this luminosity is not affected in any important way. It can be assumed that these constituents, being in close contact with the explosive crystals, are also brought to these high pressures and temperatures during the detonation.

On opening the steel bombs (20 cm.  $\times$  10 cm.) in which the first experiments were done, a light, transparent, gaseous 'cloud' was evolved, and a thin powder, consisting of tiny glassy spheres 1 to 20 microns in diameter, could be obtained from the internal wall of the bomb by brushing. Such clouds when confined in small vessels (5 cm.<sup>3</sup>) at a pressure of about 4000 kg./cm.<sup>2</sup> and between 400° and 700° C, for 4 to 30 days, lead to the direct genesis of numerous minerals. To maintain the gaseous matter without loss under high pressures and high temperatures, steel blocks (150 mm. long and 80 mm. wide) were used (Fig. 1, 2). Through the centre of these there was a cylindrical hole 12 mm. in diam. and 120 mm. in depth. A thread was cut inside the upper 30 mm. in order to take a fastening bolt. The close joint was secured by enclosing it in three tubes, the inner one being the experimental chamber. These tubes were closed at one end and arranged to be opposite each other; they were driven into the hole of the bomb and a copper joint on a steel shoulder received the pressure strain of the bolt during fastening. The effect of the shock waves through the tubes produces a tightening of the joints.

The steel bomb was filled and closed and placed in an electric furnace. The weight of Hexogene employed was 3 g., and that of the mixed powders 0.8 g. To ensure detonation of the Hexogene before decomposition, when the steel bomb was placed in the already heated furnace, it was necessary to place, at the bottom of the charge, a few mg. of a priming explosive (metanitraniline perchlorate) as a detonator. The detonation took place at 150° C, 5-10 min. after placing the bomb in the furnace. It has been possible not only to maintain a high temperature but also to anneal in steps of decreasing temperature without any loss of pressure. Unfortunately all the different steels which were used became permeable to the gases at about 710°-720° and the pressure could not be maintained at higher temperatures. In recent experiments, however, an alloy containing 80 % Ni and 20 % Cr was used and we have succeeded in maintaining the pressure up to 850° C.



It proved impossible to open the bomb by unscrewing the bolt after a long firing and slow cooling, consequently a small hole was first carefully bored in the side of the bomb to enable the slow escape of the confined gases, thereby preventing accidents and also the dispersal of the newly produced minerals. The massive metal of the bomb was then sawn across near the upper or the lower part of the experimental chamber; afterwards, by means of a lathe, one or two cm. lengths of the inner tubes were exposed. The end of the tubes was then cautiously cut. The new crystals were then easy to observe using a strong light under the microscope. Some porcellanic, partially vitreous coatings have sometimes been observed on the more or less corroded and fused surface of the metal. Thin sections of these have been prepared and they displayed numerous fibrous spherulites and 'micro-lites.' On these coatings or directly on the metal, often at the upper part of the experimental chamber, isolated or rosette-like aggregated crystals are produced and also bows of pyramidal quartz (0.25 to 1.5 mm.), of feldspars, orthoclase, albite, anorthite; isolated crystals of orthoclase, well developed on the (110) face and about 200 microns in diameter, biotite lamellæ, magnetite and magnetic nickel crystals, etc. (Fig. 3-6).

Fragments of diamond dust were corroded in the detonation and a white pumice deposited on the upper wall, but this disappeared after a fortnight!

The metallic wall of the experimental chamber, due to the impact of the shock waves, was always superficially fused and even gasified. For instance, copper was found in the form of extremely fine drops or as *cristaux natifs* even in the interior of the crystals; and platinum was observed to have crystallized in a perfect cubic form.

To this list can be added the crystal genesis of salts (carbonates, calcite, smithsonite, cerusite, etc., and nitrates) which are obtained in the last phases of cooling.

The genesis brought about in this way shows the importance of pneumatolysis in the formation of crystalline rocks. Whether we consider superficial phenomena at the birth of our planet, or more recent phenomena underground or in volcanoes, we can suppose that pneumatolysis has been an important factor.

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## FACTORS GOVERNING THE GROWTH OF CRYSTALLINE SILICATES

BY R. M. BARRER

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**1. Introduction.** Many silicates such as kaolin, bentonite, mica, quartz, vermiculite or asbestos have important uses in industry, as have synthetic minerals, including artificial gemstones, ultramarines, insulators, ceramic ferromagnetics and ceramic conductors of spinel type. Both natural and synthetic minerals now of academic interest only may in the future become industrially important. The present paper will outline synthetic methods—largely imitative of natural geochemical processes—which have been successful in growing crystalline silicates; and will indicate some of the factors which appear to govern the nature of the crystals grown. Such studies have much to receive from and to give to mineralogy and geology.

**2. Methods of Growing Crystalline Minerals.** There are at least five different methods by which silicates and other minerals may be grown:

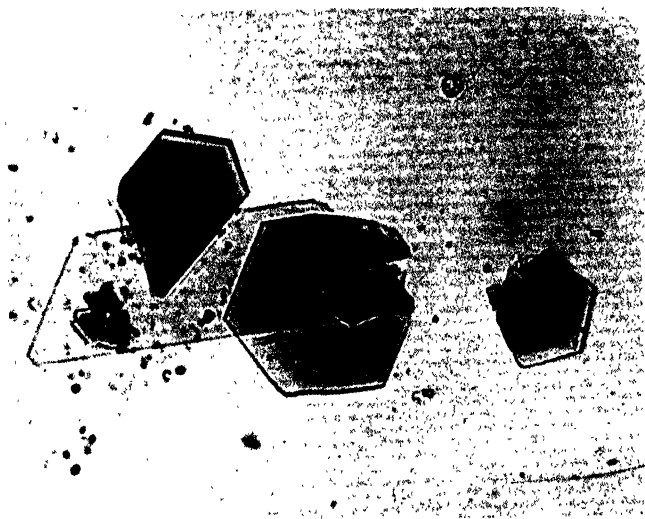


FIG. 3.—Thin greenish-black plates of biotite  $\times 400$ ; bomb annealed for 15 days at  $680^{\circ}\text{C}$  after initial explosion of the mixture,  $\text{SiO}_2$  33 %,  $\text{Al}_2\text{O}_3$  13 %,  $\text{Fe}_2\text{O}_3$  15 %,  $\text{MgO}$  16 %,  $\text{K}_2\text{CO}_3$  10 %,  $\text{K}_2\text{SiF}_6$  17 % with Hexogene.

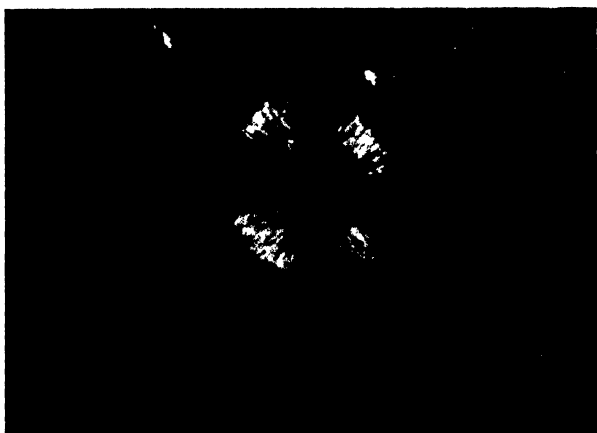


FIG. 4.—Biotite growth on a copper drop (see Fig. 3 for details).



FIG. 5.—Crystals of anorthite  $\times 100$ . Bomb annealed for 7 days at  $560^{\circ}\text{C}$  after explosion of the mixture 0.12 g.  $\text{SiO}_2$ , 0.05 g.  $\text{Al}_2\text{O}_3$ , 0.08 g.  $\text{CaCO}_3$  with Hexogene.

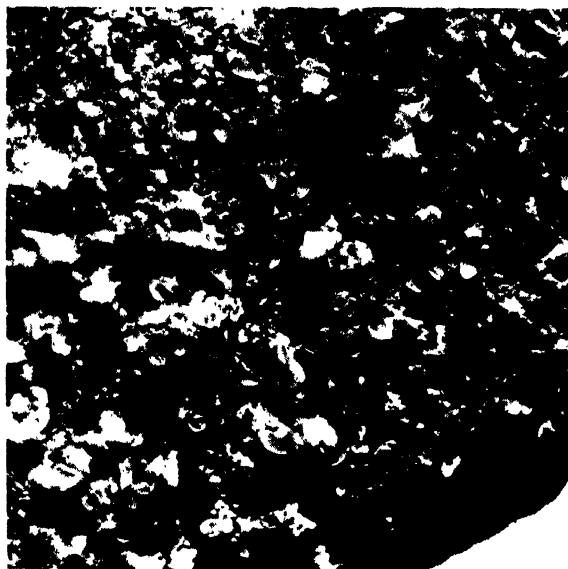
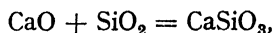
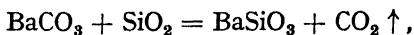


FIG. 6.—Quartz crystals (1 mm. long) on 'conglomerate.'

by sintering reactions, by crystallization from the vapour phase and by pyrolytic, high-pressure or hydrothermal crystallization from natural or artificial magmas.

(i) **SINTERING REACTIONS.** Numerous syntheses of minerals by sintering have been recorded.<sup>1</sup> The rate of reaction, which may become large well below the fusion point of the reactants, often depends on rates of ionic diffusion in one or more of the solid phases. Not only may the kinetics of formation of new species be followed quantitatively in many cases,\* but also crystal growth in a single species can be followed.<sup>4</sup> The crystals are usually small, and a polycrystalline mosaic results. The rates of such processes are sometimes greatly modified by an ambient gas. Thus in the reactions,<sup>5</sup>



accelerations of 22-fold and 8.5-fold were noted in the presence of water vapour, the effect being manifested on the term  $k_0$  only, in the rate constant,  $k = k_0 e^{-E/RT}$ . Here is a simple example of water acting as mineralizer (loc. cit.).

Sintering reactions are valuable in providing quantitative information on rate constants, energies of activation, and of the influence of grain size, compacting pressures and gas atmospheres on the kinetics. They therefore give information concerning the reaction mechanism.<sup>6</sup> The method, however, is less suited for growing large, single crystals.

In nature, metamorphoses in sedimentary and other rocks, which have become rather deeply buried and so subjected to pressure at intermediate temperatures, may take place by sintering reactions. The formation of garnet is characteristic of such metamorphism. This mineral being of considerable density, its formation under pressure is compatible with the Le Chatelier-Braun principle of mobile equilibrium.

(ii) **VAPOUR-PHASE CRYSTALLIZATION.** Good crystals of ammonium chloride can easily be grown from its vapour, and there is little doubt that many minerals could also be formed in a similar way, provided sufficiently high but controlled temperature conditions are achieved. Frankel<sup>7</sup> has produced small crystals of cassiterite ( $\text{SnO}_2$ ) by volatilization. Doelter<sup>8</sup> reported the preparation of enstatite and sillimanite at very high temperatures and probably by volatilization.

<sup>1</sup> Two articles which review such reactions are by Taylor (*J. Amer. Ceram. Soc.*, 1934, **17**, 155) and by Cohn (*Chem. Rev.*, 1948, **42**, 527).

<sup>2</sup> Weyl, *Tonind.-Ztg.*, 1929, **53**, 559.

<sup>3</sup> Taylor, *Z. physik. Chem. B*, 1930, **9**, 241.

<sup>4</sup> Taylor, ref. <sup>1</sup> ( $\text{Al}_2\text{O}_3$ ); Foex, *International Colloquium on Reactions in the Solid State* ( $\text{ThO}_2$ ) (Paris, October, 1948).

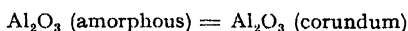
<sup>5</sup> *Idem*, ref. <sup>1</sup>. Also Jander and Stamm, *Z. anorg. Chem.*, 1930, **190**, 65.

<sup>6</sup> Cp. Serin and Ellickson, *J. Chem. Physics*, 1941, **9**, 742.

<sup>7</sup> *Miner. Mag.*, 1947, **28**, 111.

<sup>8</sup> *Mineralchemie* (Steinkopf, 1912), Vol. I, p. 601.

\* The reaction between  $\text{BaO}$  and  $\text{Al}_2\text{O}_3 \cdot 2\text{SiO}_2$  at  $357^\circ \text{C}$  may go sufficiently rapidly, according to Taylor,<sup>1</sup> to give a heat effect. That between finely divided  $\text{BaO}$  and  $\text{CuSO}_4$  in admixture may be complete in less than a second at  $346^\circ$ . The processes  $\text{MgO} + \text{Fe}_2\text{O}_3 = \text{MgFe}_2\text{O}_4$ ,<sup>2</sup>  $\text{ZnO} + \text{TiO}_2 = \text{ZnTiO}_3$ ,<sup>3</sup>  $2\text{NiO} + \text{SiO}_2 = \text{Ni}_2\text{SiO}_4$ ,<sup>4</sup> are all completed in several hours some hundreds of degrees below the eutectic temperatures of the systems involved. The reaction<sup>1</sup>



occurred only slowly at  $850^\circ \text{C}$ , giving  $< 10\%$  conversion in a specific time. In the same time, but in the presence of  $\text{CaF}_2$ , 100% crystallization was realized (loc. cit.).

Minerals may also be formed by the action of vapours upon solids. In glass-making furnaces alkali metal oxide vapours may attack furnace linings. Many processes of cation exchange can be carried out by heating zeolites with  $\text{NH}_4\text{Cl}$  vapour.<sup>9</sup>  $\text{SiF}_4$  and superheated water vapour may interact to give quartz crystals,<sup>10</sup> while  $\text{SiCl}_4$  (or  $\text{SiF}_4$ ) and water vapour interact with salts such as  $\text{MgCl}_2$  to give silicates.<sup>8</sup> Emanations from hot igneous intrusions may penetrate the surrounding country rock and react with these rocks to form important mineral deposits; or on cooling some distance from their place of origin these emanations may precipitate other species held in solution at high temperatures.<sup>11</sup>

(iii) PYROLYTIC CRYSTALLIZATION. In this method the crystals are grown by cooling a melt. An extensive literature deals with phase diagrams of systems of interest in glass-making, refractories and ceramics industries.<sup>12</sup> In addition, however, many other minerals have been grown pyrolytically and the method has proved valuable for producing large single crystals. Cooling from one end of the melt in a slight progressively moving thermal gradient has been very successful in yielding crystals for optical purposes (cp. this Discussion).

In silicate chemistry difficulties arise in growing large crystals because of the very great viscosity of the melt. Nucleation sets in at a rate which at first increases and then decreases with degree of undercooling, but subsequent growth is slow in the viscous magma, and usually only small crystals result. For too rapid cooling the whole mass sets to a glass. One of the earlier attempts at mineral growing from a substantial volume of a multi-component melt\* was made by Morowicz,<sup>13</sup> who lowered the temperature of the melt at ten-hour intervals from about  $1600^\circ$  to  $800^\circ$  C. He was able to prepare some larger crystals, and reported corundum, magnetite, iron glance, pyroxenes and wollastonite. Additions of  $\text{CaSO}_4$ ,  $\text{Na}_2\text{SO}_4$  and  $\text{NaCl}$  caused nosean, hauyne and sodalite to appear, and in the presence of a little tungstic acid small quartz crystals were found.

Crystallization of magmas follows the fairly definite pattern shown below, as far as some species at least are concerned.<sup>14</sup>

(iv) HIGH-PRESSURE CRYSTALLIZATION. During crystallization a melt is sometimes exposed simultaneously to heat and great pressure. Natural crystallizations of deep-seated igneous rocks often occur under such conditions.† Laboratory imitations of pneumatolytic high-pressure conditions have been obtained by Wyart and Michel-Lévy (this Discussion) and successful growth of small crystals of species such as feldspars, quartz, topaz and cryolite reported.<sup>15</sup>

\* This method appears to have been originated by Clark and Steiger, *Amer. J. Sci.*, 1899, **8** (iv), 245; 1900, **9** (iv), 117, 345; *Z. anorg. Chem.*, 1900, **23**, 135.

<sup>10</sup> Baur, *Z. physik. Chem.*, 1904, **48**, 483.

<sup>11</sup> Morey, *Carnegie Inst. Washington Publ.* No. 501, 1938, p. 49.

<sup>12</sup> Norton, *Refractories*, 1942 (McGraw-Hill Book Co.).

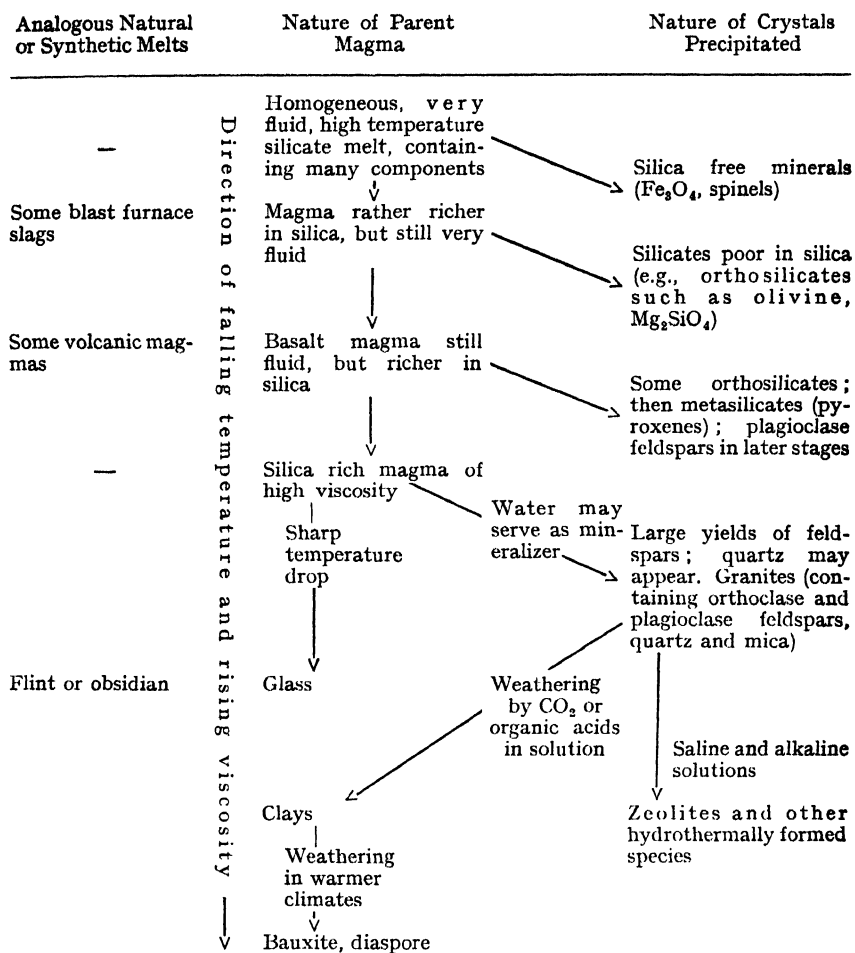
<sup>13</sup> *Tsch. min. Mit.*, 1899, **18**, 20.

<sup>14</sup> Cp., for example, Morey, *J. Amer. Ceram. Soc.*, 1937, **20**, 283.

<sup>15</sup> Michel-Lévy and Wyart, *Compt. rend.*, 1938, **206**, 261; 1939, **208**, 1030 and 1594; 1940, **210**, 733; 1941, **212**, 89; *Bull. Soc. franc. Miner.*, 1947, **70**, 164 and 168; *Mém. Soc. Géol. France*, 1947, **26**, No. 55.

\* In imitation of cooling of a natural magma.

† These have cooled very slowly between about  $1400^\circ$  and  $400^\circ$ , at pressures as high as 5 to 10 tons per sq. in., and although the crystals may vary in chemical composition they exhibit fairly uniform physical characteristics. The rocks are wholly crystalline and the crystals are large, sometimes several centimetres or more, in diameter. The range in crystal sizes is not great, perhaps because tiny crystallites have aggregated under pressure, or been preferentially consumed by the larger crystals as a result of their higher surface free energy.



(v) **HYDROTHERMAL CRYSTALLIZATION.** Water in considerable amount is essential in true hydrothermal crystallizations, which usually occur below  $600^\circ$ , often under a high pressure of water vapour so that they are in some measure pneumatolytic also. In many of the crystals the water plays a structural role in the lattice; but anhydrous crystals are also easily grown (e.g., feldspars or quartz). In general, the proportion of hydrous to anhydrous species decreases as the temperature of crystallization is raised. In addition to its structural role, the water by its solvent action upon the other components may promote their dissolution, mixing and recrystallization. The solubilities of silicates are normally small and difficult to determine.\* They depend *inter alia* upon the pH of the medium, and the process of solution may occur with chemical decomposition. Nevertheless, many hydrothermal crystallizations occur reproducibly under standard conditions and may.

<sup>18</sup> *Rec. trav. chim.*, 1935, **54**, 129.

\* For example, no very satisfactory quantitative data appear even in the simpler cases of quartz and silica (cp. v. Nieuwenberg and v. Zon<sup>19</sup>).

follow similar principles to those governing precipitation of simple salts from their solutions (e.g., analcite, mordenite and quartz<sup>17</sup>).

The hydrothermal technique of heating the aqueous mixture in autoclaves has been modified for some clay syntheses by circulating carbonic or other mildly acid solutions over alkali-rich mixtures at suitably elevated temperatures.<sup>17a</sup>

The same mineral may crystallize using several of the methods described. A few examples are given in Table I. It is interesting that there is reasonable parallelism between syntheses of these minerals and their paragenesis in nature; other examples of this parallelism could be given. A critical attitude towards reported syntheses is necessary where inadequate identification has been given. For example, a claim to have made the rare zeolite faujasite<sup>18</sup> is probably incorrect; the species formed is considered to be hieratite ( $K_2SiF_6$ ), and was due to the presence of hydrofluoric acid in the silica gel used. Reported preparations of chabazite also seem doubtful<sup>19</sup>; repetitions by the writer of some of the "preparations" described in the literature yielded species which were definitely not chabazite. Many other doubtful claims have been made.

**Crystal Dimensions.** Natural crystals often greatly exceed in size those grown synthetically. A crystal of spodumene has been found over forty feet long weighing more than forty tons; a crystal of beryl weighing nineteen tons, eighteen feet long and four feet in diameter (compared with hydrothermally grown crystals weighing 0.2 g.<sup>20</sup>); and a "book" of mica with leaves twelve feet in diameter (compared with pyrolytic synthetic mica of about  $3\frac{1}{2}$  inches in diameter). Crystals of feldspars of four or five feet have been found in pegmatite veins, compared, for example, with hydrothermally grown albite crystals of 0.1 to 0.2 mm. in length.<sup>21</sup> Natural asbestos fibres may have lengths as great as 15 cm. (chrysotile) or even 25 cm. (amosite). Pyrolytically crystallized hornblende asbestos fibres have been grown 0.4 cm. long.<sup>22</sup>

Many fine natural crystals have been formed hydrothermally, such as zeolites or quartz. By contrast, synthetic analcite<sup>23</sup> crystals up to 0.1 mm. diameter represent at present a good hydrothermal growth for a zeolite. Quartz, on the other hand, forms comparatively large crystals from mineralizing solutions. A number of syntheses have yielded crystals from 0.1 to 2 mm. in length, and substantial growths have been obtained on seed crystals suspended in the mineralizing solution. For example, a 3.3 g. specimen increased by 2 g. in four days; and growth rates of 48 mg. cm<sup>-2</sup> day<sup>-1</sup> have recently been reported, with a total deposition of quartz of 6.9 g.<sup>24</sup> By pneumatolytic growth, synthetic quartz crystals of 1.5 by 0.65 mm. have been formed.<sup>15</sup>

One reason why the largest natural crystals may greatly exceed synthetic silicate crystals in size is undoubtedly the time factor, although in part this factor has been overcome by the use of mineralizers. Also where there has to be a heating period during which thermal conditions in the autoclave

<sup>17</sup> Barrer, *J. Chem. Soc.*, 1948, 2158. *Nature*, 1946, **157**, 734; and also this paper, § 3.

<sup>17a</sup> Norton, *Amer. Miner.*, 1937, **22**, 1.

<sup>18</sup> Baur, *Z. anorg. Chem.*, 1911, **72**, 119.

<sup>19</sup> Summarized by Mellor, *Treatise on Inorganic and Theoretical Chemistry*, Vol. VI, p. 729.

<sup>20</sup> Nacken, cited by Van Praagh, ref. <sup>24</sup>.

<sup>21</sup> Friedel and Sarasin, *Compt. rend.*, 1883, **97**, 290.

<sup>22</sup> Scheumann, *Fort. Miner. Krist. Petr.*, 1933, **17**, 69. Scheumann and Ludke, *Ber. Verhand. Sachs. Akad. Wiss. (Leipzig, Math-Phys. Kl.)*, 1933, **85**, 273.

<sup>23</sup> E.g., Straub, *Ind. Eng. Chem.*, 1936, **28**, 113; and also this paper, § 3.

<sup>24</sup> See, e.g., Hale, *Science*, 1948, **107**, 393, and Van Praagh, *Geol. Mag.*, 1947, **84**, 98.

TABLE I

SUCCESSFUL SYNTHESSES OF TYPICAL SILICATE CRYSTALS \*

Crystal Types	Hydrothermal Growth	Pyrolytic Growth	High-pressure Pneumatolytic Growth	Growth by Sintering	Growth from Vapour Phase
Clays	Kaolinite <sup>25 26 27 28 29 30</sup> Dickite <sup>28 29</sup> Beidellite <sup>28 29</sup> Sericite <sup>29 27</sup> Nontronite <sup>28</sup> Montmorillonite <sup>29 27</sup>	—	—	—	—
Micas	Muscovite <sup>26</sup>	Phlogopite <sup>31 32</sup>	—	—	—
Zeolites	Analcite <sup>23</sup> Mordenite <sup>33</sup> Harmotome <sup>34</sup>	—	—	—	—
Sodalite-Hauyne minerals	Sodalite <sup>35 36</sup> Cancrinite <sup>36</sup> Nosean <sup>36</sup>	Sodalite <sup>13</sup> Nosean <sup>13</sup> Hauyne <sup>13</sup>	—	—	—
Crystal forms of silica	Quartz <sup>24</sup> Cristobalite <sup>37 38</sup> —	Quartz <sup>39</sup> Cristobalite <sup>39</sup> Tridymite <sup>39</sup>	Quartz <sup>15</sup> — —	— — —	— Cristobalite <sup>40</sup> —
Feldspars	Albite <sup>41</sup> Orthoclase <sup>41 42</sup> —	Albite <sup>43</sup> Orthoclase <sup>43</sup> —	Albite <sup>15</sup> Orthoclase <sup>15</sup> Anorthite <sup>15</sup>	Albite <sup>44</sup> — Anorthite <sup>45</sup>	— — —

<sup>25</sup> Schwarz and Trageser, *Naturwiss.*, 1935, **23**, 512.<sup>26</sup> Gruner, *Econ. Geol.*, 1944, **39** (8), 578.<sup>27</sup> Noll, *Centr. Miner.*, 1934, 80; *Miner. Petr. Mitt.*, 1934, **45**, 175; 1936, **48**, 210; *Neues Jahrb. Miner. Geol., Beil. Bd.*, 1935, **70**, 65. *Chem. Erde*, 1936, **10**, 129.<sup>28</sup> Ewell and Insley, *J. Res. Nat. Bur. Stand.*, 1935, **15**, 173 (RP 819).<sup>29</sup> Norton, *Amer. Miner.*, 1937, **22**, 1; 1939, **24**, 1; 1941, **26**, 1.<sup>30</sup> v. Nieuwenberg and H. Pieters, *Rec. trav. chim.*, 1929, **48**, 27.<sup>31</sup> Noda and Sugiyama, *J. Soc. Chem. Ind. Japan*, 1943, **46**, 931 and 1082.<sup>32</sup> Kendall and Spraggon, *XI Int. Congr. Pure Appl. Chem.*, London, 1947.<sup>33</sup> Barrer, *J. Chem. Soc.*, 1948, 2158.<sup>34</sup> *Idem* (unpublished data).<sup>35</sup> Newman, Shartsis, Bishop and Wells, *J. Res. Nat. Bur. Stand.*, 1945, **36**, 63.<sup>36</sup> Imhoff and Burkhardt, *Ind. Eng. Chem.*, 1943, **35**, 873; also Alcock, Clark and Thurston, *J. Soc. Chem. Ind.*, 1944, **63**, 292.<sup>37</sup> Wyart, *Bull. Soc. franç. Min.*, 1943, **66**, 479.<sup>38</sup> Weil, *Compt. rend.*, 1925, **181**, 423.<sup>39</sup> Cf. Fig. 3, for example, which defines the temperature-composition limits for pyrolytic crystallization of crystalline forms of SiO<sub>2</sub> from Na<sub>2</sub>O-SiO<sub>2</sub> melts.<sup>40</sup> Greig, Merwin and Shepherd, *Amer. J. Sci.*, 1933, **25** (5), 61.<sup>41</sup> v. Nieuwenberg and Blumendahl, *Rec. trav. chim.*, 1931, **50**, 989.<sup>42</sup> Gruner, *Amer. Miner.*, 1936, **21**, 511.<sup>43</sup> See Doelter, ref. <sup>8</sup>.<sup>44</sup> See Taylor, ref. <sup>1</sup>, and Day and Allen, *Carnegie Inst., Washington*, Publ. No. 31.<sup>45</sup> Jander and Petri, *Z. Elektrochem.*, 1938, **44**, 747.

\* No attempt has been made to give all syntheses claimed. References have been chosen, however, which correspond to some of the syntheses which are not of doubtful authenticity.



change, nucleation may occur before the optimum temperature is reached. Experience suggests that there is frequently an initial shower of small, often geometrically perfect, crystals from the mineralizing solution, followed by a virtual cessation of precipitation or crystal growth. Much of the difficulty in growing large crystals by hydrothermal methods has been in sustaining crystal growth following the initial shower. In a synthesis of a zeolitic species the small crystals first formed were transferred to a similar mother liquor, and heated to the temperature of the first growth; but whereas the first growth occurred in less than two days, fifteen days' subsequent treatment caused no observable change in size.<sup>46</sup> Such effects may probably be traced to depletion of the mother liquor in one or more important constituents.

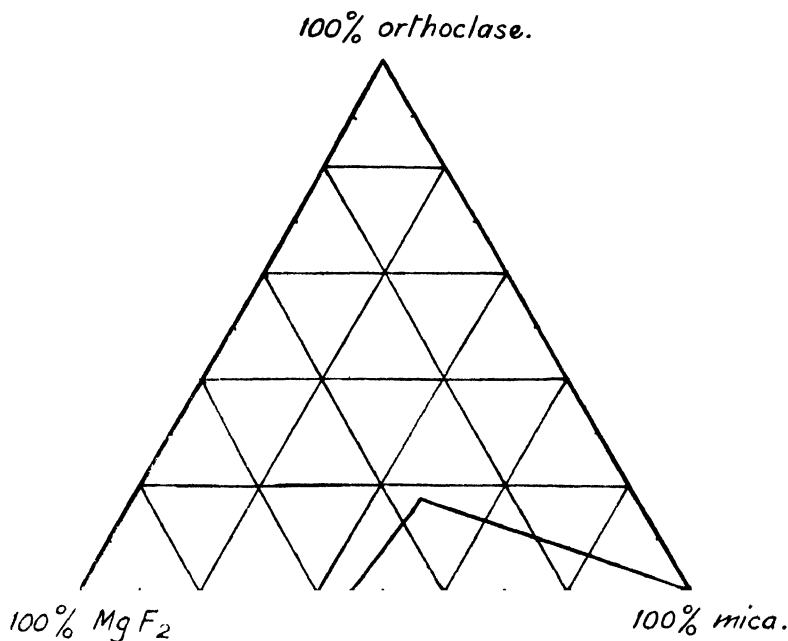


FIG. 1.—The small triangle denotes a region where good growth of phlogopite-type mica crystals is reported from melts containing  $\text{MgF}_2$ , mica ( $\text{F}_2\text{KMg}_3\text{AlSi}_3\text{O}_{10}$ ) and orthoclase ( $\text{KAlSi}_3\text{O}_8$ ). Compositions are represented by the method of Roozeboom.

**3. Variable Conditions in Growth of Crystalline Minerals.** The variable factors in growing silicate minerals include chemical composition, the presence or absence of mineralizers, temperature, pressure, time and (in hydrothermal reactions above the critical temperature) the degree of filling of the autoclave. Some of these factors will be considered.<sup>47</sup>

(i) **CHEMICAL COMPOSITION AND TEMPERATURE.** Two examples only will be given of the influence of composition and temperature upon the species crystallizing. Silicates often appear over a range of compositions, temperatures or pressures. Fig. 1 shows a composition range which is claimed to give good pyrolytic crystallization of a synthetic mica, at temperatures between  $1260^\circ$  and  $1360^\circ$  C, depending on the composition.<sup>31</sup>

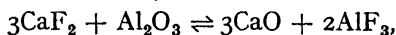
<sup>46</sup> Barrer, *J. Chem. Soc.*, 1948, 127.

<sup>47</sup> Another discussion of these factors is given by Morey and Ingerson, *Econ. Geol.*, 1937, 32, 607.

In an investigation of hydrothermal crystallization of sodium aluminosilicate gels  $\text{Na}_2\text{O} \cdot \text{Al}_2\text{O}_3 \cdot m\text{SiO}_2 + \text{aq.}$ , where  $m$  ranged from two to over twelve, three prominent species among a number of those grown under alkaline conditions were quartz, mordenite and analcite.<sup>17</sup> Quartz appeared where  $m$  was large, increasing in amount as  $m$  increased from about 8 upwards. Mordenite began to appear where  $m$  was about 8, but diminished in yield again as  $m$  approached 12; highest yields were for values of  $m$  intermediate between these extremes. Analcite crystallized in high yield for  $m = 4$  or 5, and the yield decreased rapidly for  $m < 4$ , and slowly for  $m > 5$ , although some analcite could be obtained even in the range where mordenite was the principal species. Analcite crystallized even below 200° C and was still in evidence at 310°; mordenite crystallized best in a narrower temperature range of ~265°–295°; but quartz can undoubtedly be grown over a very wide temperature range indeed (temperatures up to 390° were used but, as expected, no limits of temperature could be defined).

(ii) MINERALIZERS. In the laboratory attempts have been made to shorten the time of crystal growth by the use of crystallizing agents or mineralizers. The modes of action of mineralizers are diverse, and can best be understood by reference to specific examples.

The sintering of amorphous alumina to give corundum is greatly accelerated by  $\text{CaF}_2$ .<sup>1</sup> It is suggested that  $\text{AlF}_3$  is formed:



and that crystalline  $\text{Al}_2\text{O}_3$  then results from the reverse reaction. In this case the mineralizer acts by forming an intermediate compound. The pyrolytic crystallization of silica gel dissolved in a melt of sodium metaphosphate yielded only cristobalite in the range 700°–950° C; but from silica gel in fused sodium tungstate in the range 700°–850° C only tridymite appeared. The formation of substantial crystals of  $\text{SiO}_2 \cdot \text{P}_2\text{O}_5$  in the former instance when a large excess of free  $\text{P}_2\text{O}_5$  was also present suggests that the formation of cristobalite may have been associated with the decomposition of such an intermediate compound.<sup>48</sup>

A hydrothermal synthesis of a zeolitic species has recently been observed in which  $\text{BaCl}_2$  or  $\text{BaBr}_2$  were specific mineralizers. The salts acted as space fillers to permit and stabilize the growth of an open aluminosilicate framework. The  $\text{BaCl}_2$  or  $\text{BaBr}_2$  may subsequently be extracted from solid solution throughout the interstices of the framework to leave the salt-free zeolite.<sup>49</sup>

Not all mineralizing actions arise from intermediate compound formation. Water\* may act in pyrolytic or pneumatolytic crystallizations in two important ways: by lowering the viscosity of a magma with which it is associated; and by lowering the fusion or crystallizing temperature of crystalline species in equilibrium with the magma. At their congruent or incongruent fusion points, feldspars have viscosities of the order  $10^7$  to  $10^8$  poises (for water at 0° C the viscosity is 0.018 poises). At the much lower temperatures at which these crystals have formed in pegmatites the viscosity will be vastly larger, yet feldspathic crystals of great size can be found. It is believed that water in the magma may have lowered the viscosity and crystallizing temperature sufficiently for excellent crystal growth over geological time.<sup>11</sup>

Quantitative studies of fusion temperatures of simple silicate species in the presence of water have demonstrated the magnitude of the lowering

<sup>48</sup> Peyronel, *Z. Krist.*, 1936, **95**, 274.

\* Other volatile compounds such as HF, HCl,  $\text{SO}_2$ ,  $\text{H}_2\text{S}$ ,  $\text{NH}_3$  or  $\text{CO}_2$  may be expected to repeat some phenomena observed with water, with limitations imposed by their chemical and physical nature.

of the crystallizing temperature of given species from the water-containing melt. The crystals and a charge of water are heated in an autoclave. The resulting melt when frozen by rapid chilling consists of a brittle glass containing the water. Optical examination then reveals whether crystals are embedded in the glass or not. If there are crystals, then the system was below the fusion temperature of the species; if crystals are absent the system was above this temperature. Repetitions over a range of temperatures for each composition then fix the exact crystallizing temperature. Fig. 2 gives the fusion temperature of potassium silicates and potassium disilicates in relation to the amount of water in the crystallizing magma, and reveal how greatly these crystallizing temperatures are lowered by water.<sup>49</sup> Such great lowering is not uncommon in silicate chemistry. The crystallizing temperature of silica as cristobalite, tridymite or quartz from melts of silica

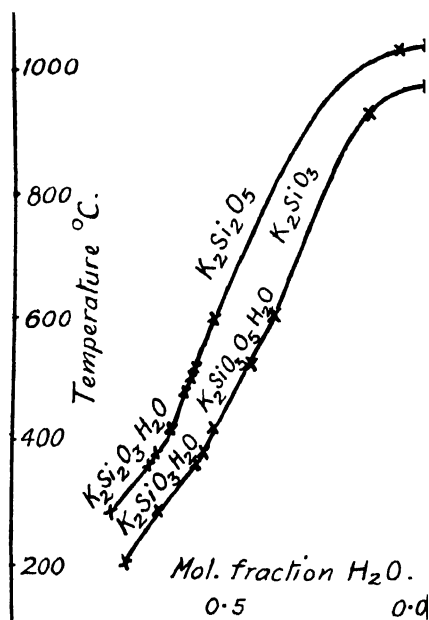


FIG. 2.—The solubility or melting point curves of the binary systems  $K_2SiO_3-H_2O$  and  $K_2Si_2O_5-H_2O$  (ref. <sup>49</sup>).

containing some sodium oxide is shown in relation to the amount of  $Na_2O$  in Fig. 3. There is a drop in the fusion temperature of the crystal forms of silica from  $1713^\circ$  to  $793^\circ$  C, and it is particularly to be noted that quartz may form from appropriate melts between  $870^\circ$  and  $793^\circ$  C.<sup>50</sup> Parallel with this lowering of the melting points following additions of  $Na_2O$  is a decrease in viscosity.

Water in a silicate magma may, at high temperatures, be expected to develop a great pressure and may in this way also influence the course of crystallization. As a hydrous magma cools from above  $1000^\circ$  C, anhydrous species commence to crystallize out, the melt in equilibrium with the crystals therefore becomes steadily richer in water, and the escaping tendency of the water at first increases. At length, after passing through a maximum the

<sup>49</sup> Morey and Fenner, *J. Amer. Chem. Soc.*, 1917, **39**, 1173.

<sup>50</sup> Morey, *J. Amer. Ceram. Soc.*, 1934, **17**, 145.

pressure beings to fall again. The final section of this falling part of the curve (between  $\sim 368^\circ\text{C}$  and room temperature) lies somewhere under the  $P$ - $T$  curve for water below its critical temperature ( $\sim 368^\circ\text{C}$ ).  $P$ - $T$  curves of water vapour (or super-critical fluid) in equilibrium with hydrous magmas and species crystallized from the magmas are given in Fig. 4 for some typical instances.<sup>49</sup>

A significant factor in hydrothermal crystallization is the pH of the solution, i.e., the  $\text{OH}^-$  ion may be an important mineralizer. Its action is exerted in part through its influence on the solubility of silica and silicates, and the biggest hydrothermally formed crystals tend to be those of species which

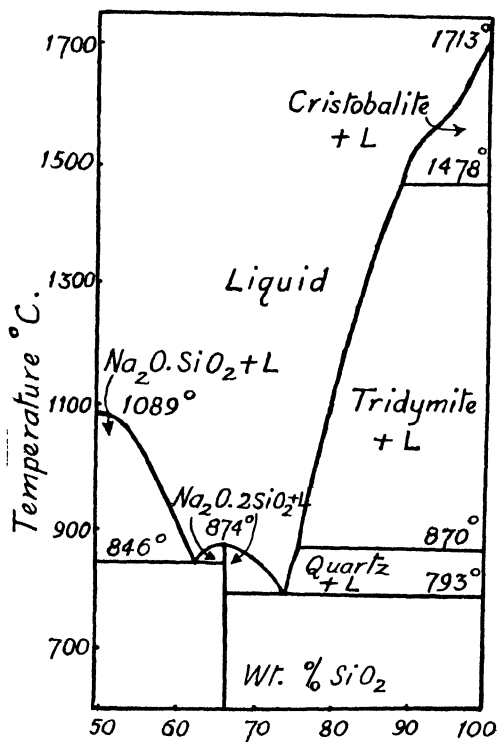


FIG. 3.—A part of the phase diagram of the  $\text{Na}_2\text{O}-\text{SiO}_2$  system showing crystals in equilibrium with melts (ref. <sup>50</sup>).

grow from alkaline media. Clay minerals on the other hand form from gels and minerals containing considerable alkali under mildly acid conditions. Possibly as a result of the low solubility of silica in acid solution, clay crystals, whether occurring naturally or made synthetically, are very small. However, it is also possible to have too high a pH. *Mordenite* formed best between about  $265^\circ$ – $295^\circ\text{C}$  and a pH between 8 and 10\* (loc. cit.); above pH 10 prolonged contact with this alkaline mother liquor resulted in the crystals first formed becoming corroded or redissolving. At a pH between 7 and 8, on the other hand, there was less satisfactory crystal growth,<sup>33</sup> although the crystals did not decompose in contact with the solution.<sup>33</sup>

(iii) MISCELLANEOUS FACTORS. When alkaline solutions act upon silica

\* The pH values given are those in the cold mother liquor after reaction.

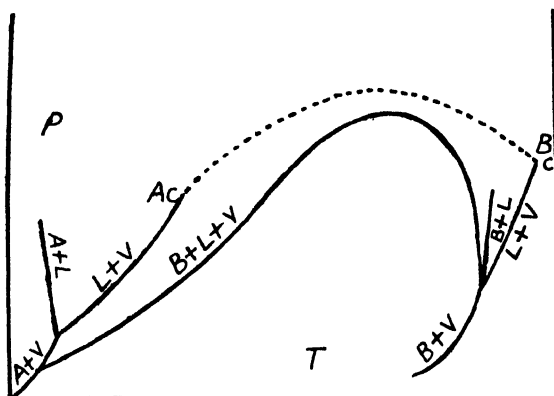


FIG. 4 a.— $P$ - $T$  curves for each of two pure components A and B in equilibrium with its vapour (V) or liquid (L). The curve  $B + L + V$  is the  $P$ - $T$  curve for B in equilibrium with liquid and vapour containing both A and B. This curve does not intersect the dotted critical curve joining the critical point  $A_c$  of A with the critical point  $B_c$  of B.

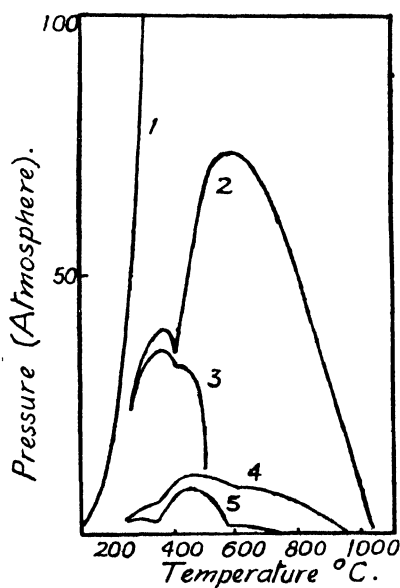


FIG. 4 b.—Equilibrium  $P$ - $T$  curves for systems containing aqueous solutions or magmas in contact with some silicate crystals (ref. <sup>49</sup>).

Curve 1 :  $P$ - $T$  curve for pure water.

Curve 2 :  $P$ - $T$  curve for  $K_2Si_2O_5$ - $H_2O$ .

Curve 3 :  $P$ - $T$  curve for  $KHSi_2O_5$ - $K_2Si_2O_5$  + Liquid + Vapour.

Curve 4 :  $P$ - $T$  curve for  $K_2SiO_3$ - $H_2O$ .

Curve 5 :  $P$ - $T$  curve for  $K_2Si_2O_5$ - $K_2SiO_3$  + Liquid + Vapour.

For a full description of all the phases present, the ternary phase diagram  $K_2SiO_3$ - $SiO_2$ - $H_2O$  must be consulted (ref. <sup>49</sup>).



FIG. 5.

(a)



(b)



(a) Ikositetrahedral synthetic analcite,  $\text{Na}_2\text{O} \cdot \text{Al}_2\text{O}_3 \cdot 4\text{SiO}_2 \cdot 2\text{H}_2\text{O}$ , grown from gel  $\text{Na}_2\text{O} \cdot \text{Al}_2\text{O}_3 \cdot 5\text{SiO}_2 + \text{aq.}$  in 42 hours at  $275^\circ \text{C.}$  ( $\times 200$ )

(b) Synthetic analcite grown from gel  $\text{Na}_2\text{O} \cdot \text{Al}_2\text{O}_3 \cdot 5\text{SiO}_2 + \text{aq.}$  in presence of NaF. Growth for 2 days at  $245^\circ \text{C.}$  The larger crystals are predominantly the cubic form of analcite. ( $\times 200$ )

glass,<sup>51</sup> or bring about crystallization of suitable aluminosilicate gels,<sup>52</sup> there is a tendency for cristobalite to separate first, but the cristobalite soon redissolves or recrystallizes and is replaced by quartz. The behaviour recalls Ostwald's law of successive transformations, in that the thermodynamically least stable polymorphic form first appears.

Another example of the operation of the time factor is shown in Gruner's conversion of montmorillonite to orthoclase (adularia). Seven days' treatment of the clay mineral at 300° C with 10 %  $\text{KHCO}_3$  solution yielded a product with a very good X-ray pattern of adularia; at 275° C the X-ray pattern of adularia had become distinct only after ten days, while at 245° C the stronger lines appeared only after six weeks.<sup>42</sup> Often, however, precipitation from a mineralizing solution occurs rapidly with little subsequent development either in the number or size of crystallites (cp. § 2).

The form of the crystals of silicate species may be modified by conditions of growth just as is the form of simpler inorganic species. Analcite often occurs as ikositetrahedra; and in this form it has been obtained by the writer in greater or less yield over a wide range of compositions of aluminosilicate gels and of temperatures (loc. cit.). However, when a gel  $\text{Na}_2\text{O} \cdot \text{Al}_2\text{O}_3 \cdot 5\text{SiO}_2 + \text{aq.}$  was crystallized in the presence of a high concentration of sodium fluoride, the analcite was obtained as cubes often showing hemihedral faces (Fig. 5 (a) and (b)). Analcite can be produced in quantity just as easily as in small amounts. A 5-kg. lot was formed by hydrothermal crystallization of  $\text{Na}_2\text{O} \cdot \text{Al}_2\text{O}_3 \cdot 4\text{SiO}_2 + \text{aq.}$  in which the crystals were of comparable dimension to those formed in the smaller lots. The crystals in all preparations were of various sizes, but some progress was made towards controlling the average diameter of crystals, since by crystallization at the lowest practicable temperatures higher yields of the smaller crystals often appeared.

## Discussion

Progress on the controlled growth of silicate and aluminosilicate crystals has been slow, owing to the difficulties inherent in high-temperature and high-pressure measurements. Future work is needed in several directions. The first of these has already been pioneered by Morey and his colleagues, who have made equilibrium studies of pressure, temperature, composition and solubility relations of simple silicates in hydrothermal systems. Phase-rule studies involving anhydrous high-temperature melts and crystals are common, but little such work yet exists for hydrothermal silicate systems, which have great geochemical and chemical interest. However, such studies have limitations, especially where there are more than three components, and also since thermodynamically less stable species often precipitate first and have the properties of greatest interest. Another approach is therefore to determine by experiment the conditions for *reproducible* formation of various species both stable and unstable, together with the complementary study of finding the conditions which control the size and form of the crystals grown.

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<sup>51</sup> Wyart, *Bull. Soc. franç. Min.*, 1943, **66**, 479.

<sup>52</sup> Taylor, *J. Chem. Soc.* (in press).



# THE HYDROTHERMAL CRYSTALLIZATION OF VITREOSIL AT CONSTANT TEMPERATURE

BY G. VAN PRAAGH

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**Historical survey.**—Silica has been crystallized in the laboratory by a number of workers and a comprehensive review of the records has been published by Kerr and Armstrong.<sup>1</sup> During the last 100 years, over 30 investigators have recorded the production of quartz in the laboratory under a great variety of conditions. The temperatures of formation have ranged from room temperature to 870° C, the pressures from 1 to 3000 atm., and the duration of the experiments from 3 hr. to 8 years. The crystals formed have ranged from a length of a few microns to 8 mm., and the length of natural crystals used as seeds has been increased to 14 mm. However, the production of a growth of more than 1 mm. is recorded in less than half a dozen cases. Almost the only early publication in this country was a report of a committee of the British Association in 1882 by Ramsay and others.<sup>2</sup> Experiments were described in this report in which various forms of silica were heated with water in a cast-iron bomb to 300°–400° C. Observations were made which recent work has substantiated and extended. As an example of the production of synthetic quartz crystals by those interested in the subject from the point of view of geological processes, the experiments of Wilson and MacGregor may be quoted.<sup>3</sup> They obtained quartz crystals up to 1 mm. long when studying the reactions between silica and sodium aluminate in the presence of water above the critical temperature. The materials were enclosed in a steel bomb, the top end of which was maintained at about 500° C for 2 months, the temperature of the bottom end being 100° lower. Small crystals of analcite and albite were identified among the products, and quartz crystals were found adhering to the silver gauze bag containing the raw material.

The most successful attempts before the war to grow crystals of quartz were those of Spezia.<sup>4</sup> Spezia used seed crystals of natural quartz suspended in a sodium metasilicate solution in a silver-lined bomb. Fragments of quartz, contained in a silver basket, were placed in the upper part of the bomb, the seed crystals being suspended just below the basket. The strength of the solution was about 2 %; the temperature in the upper part of the bomb was maintained at 320°–350° C and fell to about 200° in the lower part. Slow diffusion of the solution occurred, dissolution of the quartz taking place in the hotter region and deposition occurring on the seed at a slightly lower temperature. In one experiment, lasting 199 days, the seed crystals, consisting of two quartz plates 0.5 cm. thick, increased in length to about 2.5 cm. In another experiment, the duration of which was 5 months, perfectly clear additional growth was produced on a Japanese twin with broken terminations.

**Isothermal Crystallization of Quartz.**—During the 1939–45 war, the demand for large, untwinned crystals of quartz for use as a source of piezoelectric oscillators became heavy. Basing his work on that of Spezia,

<sup>1</sup> Kerr and Armstrong, *Bull. Geol. Soc. Amer.*, 1943, **54**, Suppl. I.

<sup>2</sup> Ramsay *et al.*, *Rep. Brit. Assn.*, 1882, 239.

<sup>3</sup> Wilson and MacGregor (in course of publication).

<sup>4</sup> Spezia, *Acad. Sci. Torino. Atti.*, 1905, **40**, 254; 1905, **41**, 158; 1908, **44**, 95.

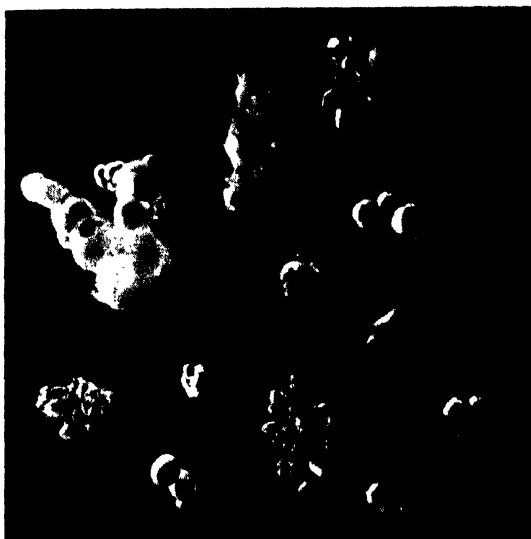


FIG. 1.—Synthetic quartz crystals and cristobalite. ( $\times 60$ )

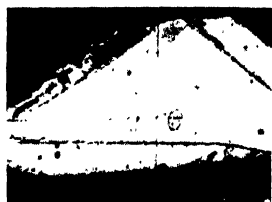


FIG. 2.—Quartz section with added growth. ( $\times 20$ )



Nacken in Germany developed techniques by which sizeable crystals could be grown in a very much shorter time than had been achieved previously. Thus, a crystal 2 cm. in length could be grown from a small seed plate 0.5 cm. long in 4 or 5 days. Work in this country proceeded along similar lines and was briefly reported by Wooster in 1946.<sup>6</sup>

Nacken<sup>6</sup> used an isothermal process in which vitreous silica was converted to  $\alpha$ -quartz at constant temperature. He was led to adopt this method through a study of the solubility of the various forms of silica in water and alkaline solutions at temperatures in the neighbourhood of the critical point. The writer has since repeated and extended some of Nacken's experiments.

It is clear from the phase diagrams of silica and the system silica-water that the isothermal transformation of vitreous silica to the crystalline forms cristobalite, tridymite and quartz is feasible. Vitreous silica is the least stable form and, below 575°C,  $\alpha$ -quartz is the most stable. Hence if vitreous silica, or one of the crystalline forms less stable than quartz, is used as the starting material, it should show a strong tendency to convert to quartz. This does in fact occur, and the transformation is easily brought about in the presence of water. In the neighbourhood of the critical point, vitreous silica is about 10 times as soluble in water as  $\alpha$ -quartz. Thus vitreous silica can be dissolved to produce a solution that is highly supersaturated with respect to quartz and will readily deposit the latter. In fact, synthesis of quartz from vitreous silica in this manner takes place so readily that one of the greatest difficulties in growing large crystals is to maintain the state of metastability and to prevent the vitreosil crystallizing *in situ* rather than on the quartz seed.

Since many of these experiments were performed above the critical temperature of water, it is evident that silica is transported through the vapour phase. Attempts have been made to measure the solubility of silica in steam at various temperatures and pressures above the critical point.<sup>7</sup> The results accord with those predicted by theory, i.e., the solubility increases with pressure and decreases with rise of temperature. There appears to be no discontinuity in the solubility curve on passing through the critical temperature. Above this temperature, the fluid phase is, of course, entirely vapour, but it is just as much a solution as an ordinary aqueous solution at room temperature. The concentration in this phase is determined by the degree of filling of the container, i.e., the fraction that is filled with liquid water at the beginning of the experiment. Unfortunately, few of the records of the synthesis of quartz include mention of the degree of filling, so that the pressure used, and hence the concentration of the vapour solution, cannot be calculated in these cases. For water, a degree of filling of about 33 % is necessary for the two phases, liquid and vapour, to become one at the critical temperature. For other degrees of filling, the system will become one phase at a lower temperature. Below 33 % filling, the single phase formed is vapour and the pressure is less than the critical; but for degrees of filling above 33 %, the single phase first formed is liquid. When the temperature is raised to the critical value, the liquid vaporizes to produce a pressure which may be greatly in excess of the critical. The importance of this factor is that, in determining the concentrations of the vapour solutions, it may influence the nature of the product that crystallizes out.

<sup>6</sup> Wooster, *Nature*, 1946, **157**, 297.

<sup>7</sup> Nacken (private communication, 1945).

<sup>7</sup> Smits, *Rec. Trav. Chim.*, 1930, **49**, 962. Van Nieuwenburg and van Zon, *ibid.*, 1935, **54**, 129. Ingerson and Morey, *Econ. Geol.*, 1940, **35**, 772.

### Experimental and Results

The equipment used by Nacken consisted of silver-lined autoclaves of various designs, with capacities varying from 30 to 300 cm.<sup>3</sup> The quartz seeds were suspended from silver wires and the vitreosil was placed on the bottom of the container. The autoclaves used by the writer are heated in an electric furnace, the temperature of which can be kept within half a degree. A number of factors can be varied, namely, the nature of the solution used, its concentration, temperature and the degree of filling of the bomb.

The effect of temperature is marked: an increase from 370° to 400° C trebles the rate of growth. The use of higher temperatures emphasizes the degree of metastability and increases the proportion of material that is devitrified *in situ*.

The effect on the growth of a range of concentrations of many solutes has been studied. For each solute there appears to be an optimum concentration that leads to a maximum amount of growth. The solutes studied include sodium carbonate, bicarbonate, borate, acetate, chloride, disodium hydrogen phosphate and alkaline ammonium fluoride. Many combinations of the variables lead to the production of quartz. Conditions giving maximum solubility of silica do not necessarily lead to maximum growth on the seed crystal. Again, high rates of growth do not give the best type of deposit, as flaws and inclusions are more likely to occur.

Under suitable conditions, the whole charge of vitreosil can be converted into quartz in a few hours. Deposition occurs on all available surfaces, only a small percentage of the whole being deposited as a clear crystalline growth on the quartz seeds. Some is deposited on the walls of the autoclave as a coherent layer, some as well-formed bi-pyramids of quartz, and the remainder is converted *in situ* into a chalcedonic mass. It will be convenient to consider the products of the crystallization of the vitreosil under three headings.

**The growth of quartz on the quartz seed crystals.**—Under favourable conditions this deposit consists of a clear crystalline growth in crystallographic continuity with the seed crystal. Fig. 2 shows a photograph of a thin section across a quartz seed with added growth, taken between crossed Nicols. The seed used had a dirty surface and the line of demarcation between the original crystal and the added quartz is thus rendered visible.

If a broken piece of natural quartz is used as the seed, the broken surfaces are healed by the added growth. Fig. 3 shows growing faces spreading over the irregular surface of the seed crystal.

The rate of growth depends on the direction of growth, and the shape of the final crystal is influenced by the nature of the solution used. The basal surface of quartz is seldom observed<sup>\*</sup>: it grows rapidly with respect to the rhombohedral surfaces and finally degenerates into the upper corner of the crystal. The prism faces usually have a lower rate of growth, the trapezohedron and trigonal pyramids grow faster and therefore remain small or disappear. These points are illustrated by an experiment of Nacken in which a sphere was cut from natural quartz and allowed to grow. Growth was rapid along the *c*-axis and positive and negative rhombohedra formed at both ends. Near the centre, a number of contiguous rhombohedral faces formed, lending a barrel-like appearance to the crystal. The original sphere weighed 3.33 g. and increased in weight by 2 g. in 4 days. Other growth tests were made with seed plates cut in different ways and proof was obtained, by an examination of the etch figures, that no spontaneous twinning occurred, e.g., a plate cut from a positive rhombohedral face grew into a positive rhombohedron, whether the lateral faces were cut along the correct crystallographic axes or not.

**The deposits formed on the surface of the container.**—In the continuous deposit formed on the walls of the crucible, minute cavities containing ingrowing crystals, reminiscent of a quartz druse as found in nature, are often formed. Small well-formed bi-pyramids of quartz are sometimes deposited on the silver wire and elsewhere. The growth of prism faces is less frequently observed. In one experiment, the quartz bi-pyramids showed a structure in their equatorial

<sup>\*</sup> Hale, *Science*, 1948, 107, 393.



FIG. 3.—Quartz growing on surface of broken seed crystal. ( $\times 20$ )

FIG. 4.—Quartz bi-pyramids. Crossed Nicols. ( $\times 60$ )

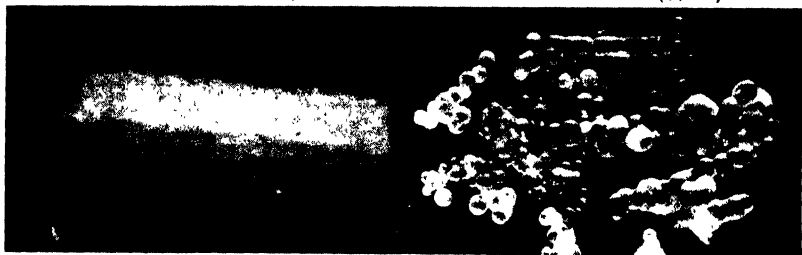


FIG. 5.—Partly devitrified silica glass showing amorphous and quartz layers. ( $\times 40$ )

FIG. 6.—Globular material formed in early stages of devitrification. ( $\times 60$ )

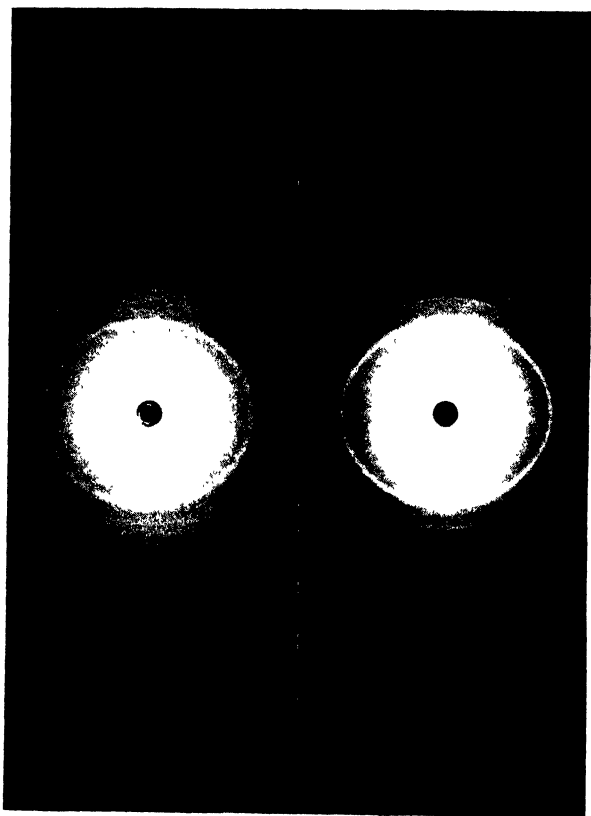


FIG. 7.—X-ray photographs of globules.



FIG. 8 (a).



FIG. 8 (b).

planes which might be described as sector twinning (Fig. 4). This observation may support the view (suggested by further evidence described below) that they had developed by growth on spherical nuclei with a radial structure.

**The devitrification of the vitreosil *in situ*.**—During an experiment, the piece of vitreosil becomes enclosed in an envelope of quartz, which, if the process of crystallization is allowed to continue long enough, extends inwards and finally replaces the whole of the vitreosil. The quartz layer is brittle and consists of an aggregate of crystal grains, many of which terminate in pyramidal faces pointing outward into the solution. If crystallization of the vitreosil has not been complete, this layer may be easily parted from the residual core of glass. The layer often consists of two parts, an outer part of quartz separated from the vitreosil by an apparently amorphous layer (Fig. 5). The material forming the latter exhibits curved surfaces and often shows a blueish opalescence. In some experiments, devitrification of the vitreosil led to the formation of aggregates of spherical particles, illustrated in Fig. 6. The diameter of the particles is of the order of 0.02 mm.; their specific gravity is 2.2 and their refractive index varies from about 1.46 to 1.48.

X-ray photographs of these particles have been kindly taken by Dr. Bannister. One sample shows what is essentially a low cristobalite pattern and, in addition, haloes which could be attributed to opal; another sample gives a photograph of high temperature cristobalite (Fig. 7).

These results form the beginning of a study of the mechanism of the crystallization of vitreosil. In general, it is clear that both quartz and cristobalite may form directly from the vitreosil, but the appearance of some of the devitrified material suggests the possibility that the cristobalite may recrystallize into quartz.

Two suggestions may be put forward to account for the spherical shape of the cristobalite particles.

(1) The silica glass may, as surface dissolution begins, aggregate into drops which then either partly or wholly crystallize. The halo shown in the X-ray photograph might be attributed to opal or to vitreosil, indicating that the drops had only partly crystallized. Wooster has observed that vitreosil flows under the conditions of these experiments and this may lend support to this view of the origin of the spherical particles.

(2) The particles may be formed by regular radial growth from a number of nuclei. Examination of the angles of contact between the spheres favours this hypothesis rather than (1).

It is interesting to compare the quartz layers formed in these experiments with the quartz crystals frequently found in cavities in natural flint. Fig. 8 shows photographs taken between crossed Nicols of thin sections of (a) devitrified vitreosil, and (b) quartz growing in a flint.

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## THE HYDROTHERMAL SYNTHESIS OF QUARTZ

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*Received 28th February, 1949*

The investigation, which we are now briefly reporting, was started late in 1942 when there was an acute shortage of crystalline quartz suitable for piezoelectric oscillators. The aim was to prepare artificial crystals of quartz large enough for commercial use.

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In common with others our first step was to repeat the experiments carried out by Spezia in 1906. In his method a high-pressure container is used containing a solution of sodium metasilicate and sodium chloride which is replenished from lumps of crystalline quartz at the upper and hotter part of the container, a seed crystal being placed near the bottom. The pressure is developed hydrothermally, the temperature of growth being somewhat below 300° C.

Spezia's results were confirmed by us but at that time his method seemed too slow and an alternative was sought. It was soon found that silica glass is readily attacked by the Spezia solution and according to the pressure-temperature conditions it either devitrifies into a profusion of quartz crystals about a millimetre long or it passes into solution. First the devitrification aspect was pursued, attempts being made to restrict nucleation so that only one or two large crystals formed. Thus silica glass was used in various special shapes, such as a rod drawn down to a narrow neck, or with various protections surrounding all but a very small area. Experiments in which the silica glass was enclosed in a steel tube were unsuccessful but they confirmed that the silica glass is strongly attacked in the vapour phase. A protective coating of a special glass came nearer to success but there were difficulties due to differential expansion. Although abandoned on account of practical difficulties, the direct crystallization approach is mentioned here as it may still merit further examination later should some new factor arise.

The other approach, in which the silica glass is used to replenish the Spezia solution as quartz is deposited on the seed crystal, led directly to the isothermal method<sup>1</sup> which is the basis of our present-day process for growing quartz. In place of Spezia's temperature difference a uniform temperature is used, the growth cycle being dependent on the much higher solubility of silica in the vitreous than in the crystalline form. It may be appropriate to observe that we have not discarded the possibility of a thermal gradient method using silica glass or perhaps silica in some other form as a raw material. However, our best results, including the artificial quartz from which we have cut satisfactory oscillator plates, have all been obtained under isothermal conditions.

### Experimental and Results

The type of autoclave used is shown in Fig. 1. In much of the earlier work they were made of mild steel except for the bolts and nuts which were of special heat-resisting steel. More recent practice is to use a special steel throughout. With routine care the mild steel lens ring provides a complete seal for pressures of the order 1000 atm. at a temperature of 360° C. The seatings on the body and lid of the autoclave are ground to conical surfaces so that with the spherically ground surfaces of the lens ring a line seal is formed. The autoclave shown has a nominal capacity of 500 ml. with a bore of 5 cm. When a new autoclave is put into service it not only must be thoroughly clean but it may be necessary to acid etch the interior walls. After the first two or three growth cycles, each of eighteen hours' duration, a closely adherent layer of polycrystalline quartz forms on these walls and it is only then that the best growth of quartz is obtained. When used in rod form the silica glass is usually suspended above the seed crystal, but when in the form of broken lumps it rests in the bottom of the autoclave. The silica glass used is of the transparent grade, translucent Vitreosil being unsuitable because it devitrifies very rapidly. Either silver or copper wire may be used for suspending the seed crystal and a copper disc is attached to the underside of the lid so that only the minimum of steel surface is exposed to the autoclave contents.

<sup>1</sup> Wooster and Wooster, *Nature*, 1946, **157**, 297.





FIG. 2.—Growth of quartz on spherical seeds.

The solution used contains sodium metasilicate, of which a typical concentration is 50 g./l., and a mineralizing substance. The function of this mineralizer which is commonly potassium acid fluoride is to improve the crystalline perfection of the deposited quartz, and it possibly brings this about by retarding the attack of the silica glass. The chemistry of the growth cycle is difficult to investigate but it is known that alkaline silico-fluorides are produced and this hint of an intermediate reaction suggests that the mineralizer is not correctly described as a catalyst. Added weight is given to this view by the high optimum concentration, which for potassium acid fluoride is about 125 g./l. The choice of mineralizer was made after several possibilities had been investigated, including a number of alkalis, alkali halides, phosphates and tungstates. It is in this use of relatively high concentrations of vehicular substance and in the use of a mineralizer that the isothermal process described here differs from the isothermal process investigated independently by Prof. Nacken in Germany during the war years.

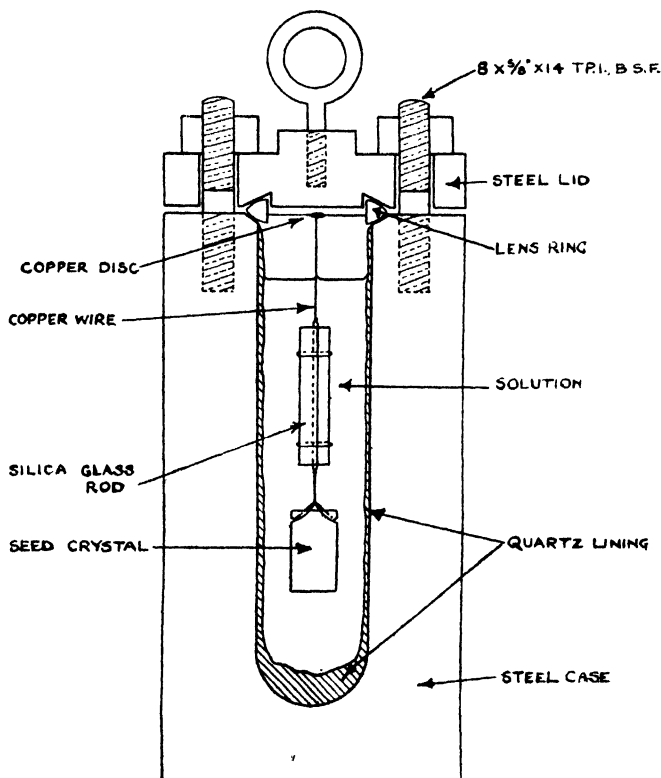


FIG. 1.—500 ml. autoclave for the hydrothermal synthesis of quartz.

The choice of the crystallographic orientation of the seed was made following experiments in which quartz was deposited on spheres of natural quartz. As shown in Fig. 2 the resultant crystal had pyramidal terminations with well-developed rhombohedral faces at both ends of a barrel-shaped prism. Further depositions on seed plates cut at various orientations supported the conclusion that plates cut parallel to the major or minor rhombohedral faces would be satisfactory as seed crystals. Much higher rates of growth are obtained on basal plane slices but it seems that growth of good crystalline quality is not obtained on such slices until complete pyramidal caps have been formed. Thus the isothermal process employs the deposition of layers of quartz on seed plates

substantially parallel to rhombohedral faces, although plates such as the BT-cut have been used which are as much as  $12^\circ$  removed from these faces.

The building-up of the synthetic quartz layer has to be accomplished in successive growing cycles of 18 hr. each, in between which the autoclave has to be recharged with raw materials. Each 18-hr. run consists of some 5 hr. up to a temperature of  $360^\circ\text{C}$  where the autoclave is held for the remaining 13 hr. before allowing to cool. In this time the normal rate of deposition for good crystalline quality is 100 mg./sq. cm. of seed surface, i.e., a total thickness increase of somewhat less than 1 mm. Thus, to obtain material for fabricating oscillator plates, entirely of synthetic quartz, about 5 successive depositions are necessary. This discontinuous feature of the process is a serious disadvantage which we are seeking to minimize or to overcome. The limiting factor is the rate of devitrification of the silica glass. There is an optimum charge of silica glass above which devitrification takes place before all the glass has dissolved. It is possible to increase the length of growth cycle to about 48 hr. with a gain in the amount of quartz deposited but, above this, further time without recharging the autoclave appears useless. A series of photographs of some of the earlier synthetic quartz specimens is shown in Fig. 3. The top row illustrates the sequence in which an entirely synthetic slice is obtained by successive depositions of good quality quartz on a seed crystal, whilst the bottom row shows typical ways in which the process may fail under incorrect conditions.

Late in 1945 our first synthetic quartz oscillator plate was mounted to operate at 150 kc./sec. but it had poor performance. Since then the isothermal process has not been modified in any important feature but the effects of the many variables have been explored in the course of several hundred experiments. Consequently the standard of reproducibility of the process has been so improved as to permit the production of synthetic quartz layers which give oscillator plates up to the standard of plates made from natural quartz. This statement needs the qualification that most of these plates have been made from R-cut material, and the experience with oscillator plates of other orientations is rather limited. Fig. 4 shows photographs of 8 Mc./sec. crystal units employing an R-cut and a BT-cut plate respectively, which have been made entirely from synthetic quartz.

The problems associated with the need for providing oscillator plates of any orientation demanded by the practical application cannot be disentangled from the problem of crystalline perfection and its dependence on seed orientation. Also, as is to be expected, the rate of quartz deposition on the seed is bound up with the quality. For example, the BT-cut oscillator plate shown in Fig. 4 was cut from a synthetic quartz layer deposited in a single run of 18 hr., during which the thickness increase was around 2 mm. In this instance the high rate of deposition has involved an appreciable lowering of crystalline quality with consequent loss in activity of the finished oscillator unit. In general a growth rate of 250 mg./sq. cm. of seed surface can be obtained reproducibly in an 18-hr. run, but only at the expense of good crystalline quality. The crystalline perfection of the synthetic quartz layer has been examined in several typical instances using various X-ray methods which have been described elsewhere.<sup>2</sup> Complementary to such an examination is the visual and microscopic inspection for mechanical faults or inclusions. We stress this part of the examination as quartz which is otherwise of a high crystalline quality may be useless for oscillator purposes owing to a single very slight flaw or bubble.

The considerations of the preceding paragraph have led the investigation more and more towards a study of the growth mechanism in the hydrothermal synthesis of quartz. Seed orientation and surface texture, solution compositions and rate of synthetic quartz deposition are the main factors under consideration. At the same time we are continuing with an investigation of alternative methods of growing quartz in parallel with the main effort on our standard process.

It is a fair statement that crystals of quartz can now be grown on a laboratory scale which make satisfactory oscillator plates. Apart from the

<sup>2</sup> Wooster and Macdonald, *Acta Cryst.*, 1948, 1, 49.

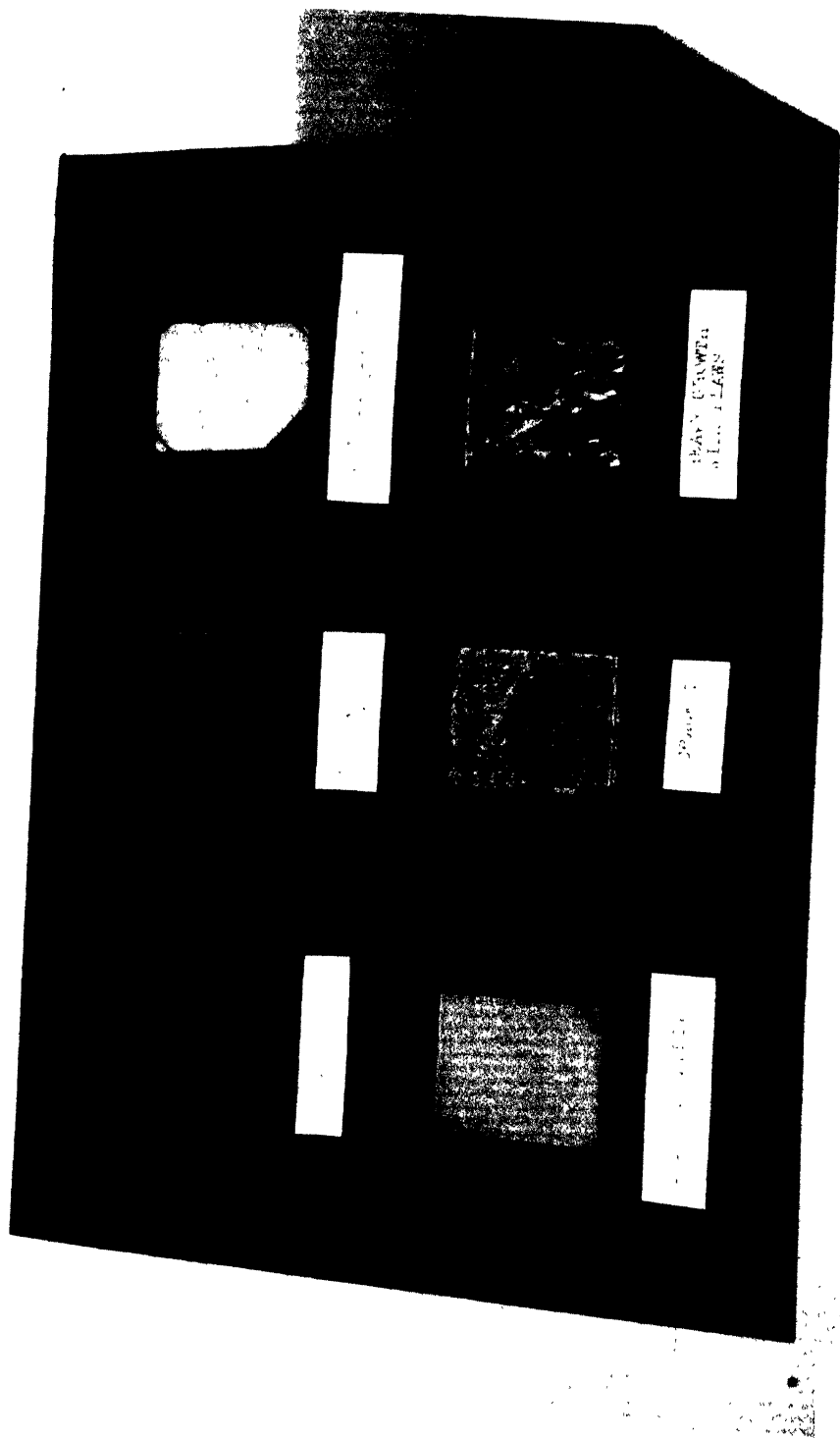


FIG. 3.—Examples of early synthetic quartz specimens.

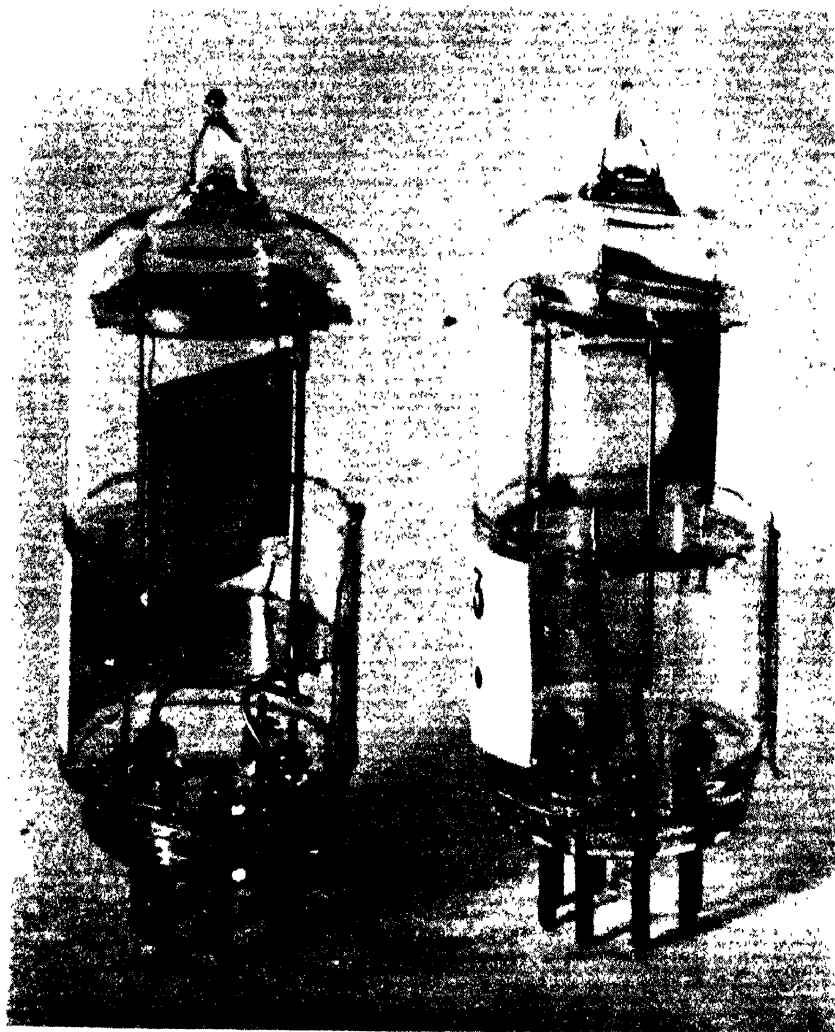


FIG. 4.—Synthetic quartz 8 Mc./sec. oscillator plates. BT-cut (left), R-cut (right).







FIG. 1.—Periclase (MgO) crystals in glass. ( $\times 250$ )

practical aspect, however, valuable knowledge of the fundamentals of crystal growth is being gained. Also we have a means of preparing quartz having special properties. Thus an amethyst-like quartz has been grown using an added manganese impurity, and experiments have been conducted in the preparation of twinned quartz or quartz with lineage structure. It may well be that the investigation of such side issues is as valuable as the preparation of artificial quartz for piezoelectric use.

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## PROBLEMS OF CRYSTAL GROWTH IN BUILDING MATERIALS

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The growth of crystals is of interest in various directions in the study of building materials and structures. The present paper will be limited to problems arising in the manufacture of building materials and to some crystalline changes which arise subsequently when the material is in use.

**Crystallization at High Temperatures.**—Industrial materials such as slags and cements are produced by sintering or fusing the raw materials at high temperatures, and there is considerable technological interest in the extent and manner of crystal growth. With slags and high alumina cement the mix becomes completely molten and crystallization takes place from the liquid phase, but with Portland cement only some 20–30 % of the mix becomes liquid and crystal growth occurs both as a result of solid-liquid reactions and of direct crystallization from the liquid.

The study of the phase equilibria diagrams is an essential feature of work on crystal growth in such materials for it enables the order of appearance on cooling of different crystals to be defined and the effect of departures from equilibrium to be traced. The complicated crystallization paths which can arise in polycomponent silicate systems have been discussed by various authors.<sup>1,2</sup> They frequently involve disappearing phases and produce typical crystalline structures such as eutectic and peritectic patterns, corroded crystals and zoning, etc. Attention has also been drawn to the possibility of “independent crystallization” of the liquid phase taking place when the cooling process is rapid or involves two or more distinct stages.<sup>3,4</sup> The final structure in such cases of frozen equilibrium can be predicted from the phase equilibrium diagrams.<sup>5</sup>

**Crystallization from the Melt.**—A most noticeable effect in polycomponent silicate and aluminate systems is the tendency of particular compounds when crystallizing from the melt to appear as spheres (Fig. 1). This effect is not related to the symmetry, being very obvious in the case of  $2\text{CaO} \cdot \text{SiO}_2$ , which has quite low symmetry. Optically the crystals

<sup>1</sup> Bowen, *The Evolution of the Igneous Rocks* (Princeton, 1928).

<sup>2</sup> Hall and Insley, *Phase Diagrams for Ceramicists* (Amer. Ceram. Soc., 1947).

<sup>3</sup> Lea and Parker, *Phil. Trans.*, 1934, **234**, 1.

<sup>4</sup> Lea and Parker, *Building Research Tech. Paper* No. 16 (H.M. Stationery Office, 1935).

<sup>5</sup> Parker and Nurse, *J. Soc. Chem. Ind.*, 1939, **58**, 255.

appear to be single individuals and it is only at a later stage when the crystals are larger, or under conditions of slower growth, that normal faces and forms appear. It is generally considered <sup>6</sup> that curved or vicinal faces arise when steep concentration gradients exist in the solution or when the rate of diffusion is low. This can result from a high viscosity of the melt or from a high rate of crystallization. Spherical growth does not occur in melts of high silica content such that glasses are readily formed, but it is characteristic of compounds such as  $\text{CaO}$ ,  $\text{MgO}$ ,  $2\text{CaO}.\text{SiO}_2$  and  $3\text{CaO}.\text{MgO}.2\text{SiO}_2$ , and to a lesser extent  $3\text{CaO}.\text{Al}_2\text{O}_3$  and  $5\text{CaO}.3\text{Al}_2\text{O}_3$ , which either do not form glasses or are easily devitrified. Spinel, which readily forms a glass, always develops as minute octahedra (Fig. 2). If instability of the glassy state is to be associated with a high crystallization rate it would appear that the latter, as well as the viscosity of the melt, is an important factor determining the growth of spherical crystals.

Growth of crystals by a solid-liquid reaction does not lead to spherical forms. Thus  $3\text{CaO}.\text{SiO}_2$  formed in this way shows its true symmetry, but small crystals grown from a suitable melt are often spherical.

Minerals differ much in their rate of crystallization, and this frequently leads to complication of the crystallization path and to changes in the form of crystal growth. Where an incongruently melting compound occurs in a system the phase first crystallizing may react with the liquid at a definite temperature to form a new species. Frequently the new crystal grows at such a rate that the dissolving primary crystals are enveloped and excluded from contact with liquid, so that on complete solidification the second phase may still contain inclusions of the first. Typical cases are inclusions of  $\text{CaO}$  in  $3\text{CaO}.\text{Al}_2\text{O}_3$  (Fig. 3) and  $\text{MgO}$  in monticellite. If such a formation is held for a sufficient length of time at a temperature just below the saturation temperature of the second phase the inclusions disappear. The rate of diffusion in solid phases at such temperatures must, therefore, be quite high.

**Growth of Single Crystals.**—Work on the X-ray structure of cement minerals has been hindered in the past because the low symmetry necessitates single crystal determinations, and methods for growing the crystals had not been devised. Many of the minerals melt incongruently or, as in the case of  $3\text{CaO}.\text{SiO}_2$  decompose below the melting point, and cannot, therefore, be grown from melts of their own composition by conventional methods. Attempts to encourage crystallization by the addition of mineralizers have sometimes been successful, but many failed because not enough was known of the crystallization paths in complex systems. Le Chatelier, for instance, was unable to crystallize  $3\text{CaO}.\text{SiO}_2$  from melt containing  $\text{CaCl}_2$ . This subject has been taken up again by one of the present authors <sup>7</sup> and single crystals of pure  $3\text{CaO}.\text{SiO}_2$  up to 1 mm. and  $3\text{CaO}.\text{SiO}_2$  solid solution up to 5 mm. in length have been grown. Twinned crystals of  $\beta$   $2\text{CaO}.\text{SiO}_2$  up to 1 cm. long have also been obtained (Fig. 4 and 5). The method used for  $3\text{CaO}.\text{SiO}_2$  is briefly as follows.

Fig. 6 is a schematic diagram of the system  $\text{CaO} - 2\text{CaO}.\text{SiO}_2 - \text{CaCl}_2$ . A composition such as denoted by B (35 %  $3\text{CaO}.\text{SiO}_2$ , 35 %  $\gamma$   $2\text{CaO}.\text{SiO}_2$ , 30 %  $\text{CaCl}_2$ ) was made up from the previously reacted silicates. A total weight of 5 g. of the mixture was heated in the electric muffle at a temperature of  $1500^\circ\text{C}$  in a platinum crucible 2.5 cm. diam. and 2.5 cm. tall. After a period of about 4 hr. most of the chloride had evaporated; the melt was then cooled in air and "dusted" owing to the  $\beta$ - $\gamma$  inversion of  $2\text{CaO}.\text{SiO}_2$ .

<sup>6</sup> Wells, *Ann. Reports*, 1946, 84.

<sup>7</sup> Nurse, *21st Cong. Ind. Chem.* (Brussels, 1948).



FIG. 2.—Spinel ( $\text{MgO}.\text{Al}_2\text{O}_3$ ) crystals in glass. ( $\times 250$ )

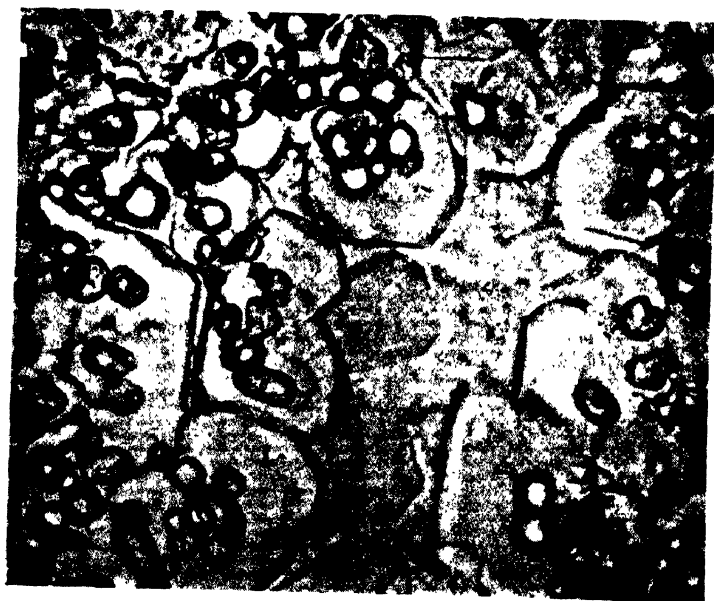


FIG. 3.—Inclusions of  $\text{CaO}$  in  $3\text{CaO}.\text{Al}_2\text{O}_2$ . ( $\times 250$ )



FIG. 4.—Single crystals of  $3\text{CaO} \cdot \text{SiO}_2$ . ( $\times 20$ )

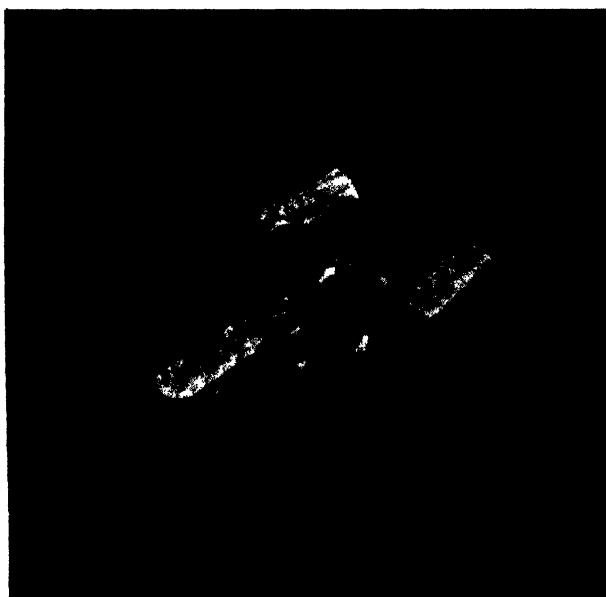


FIG. 5.—Twinned crystals of  $\beta\text{-}2\text{CaO} \cdot \text{SiO}_2$ . ( $\times 20$ )

The large crystals of  $3\text{CaO} \cdot \text{SiO}_2$  were then separated by washing with alcohol on a 300-mesh sieve.

Neglecting the decomposition and oxidation of  $\text{CaCl}_2$  into  $\text{CaO}$  and  $\text{Cl}_2$ , the melt composition follows the line BA in Fig. 6 and eventually passes into the  $3\text{CaO} \cdot \text{SiO}_2$  primary phase field. Crystals of  $3\text{CaO} \cdot \text{SiO}_2$  begin to form when the  $1500^\circ\text{C}$  isotherm is reached and since the melt is then losing both  $\text{CaCl}_2$  and  $3\text{CaO} \cdot \text{SiO}_2$ , the melt composition follows the isotherm towards the  $2\text{CaO} \cdot \text{SiO}_2$  field. When all the chloride has evaporated the melt has composition A and consists of large crystals of  $3\text{CaO} \cdot \text{SiO}_2$  and  $2\text{CaO} \cdot \text{SiO}_2$ . Similar methods have been used to prepare single crystals of a new compound  $3\text{SrO} \cdot \text{SiO}_2$  from the oxides and  $\text{SrCl}_2$ . It is not isomorphous with  $3\text{CaO} \cdot \text{SiO}_2$ .<sup>8</sup>

The successful preparation of spinel boules in the Verneuil furnace suggests that it should be possible to grow single crystals of congruently melting compounds, such as  $\text{CaO} \cdot \text{Al}_2\text{O}_3$ ,  $12\text{CaO} \cdot 7\text{Al}_2\text{O}_3$ ,  $4\text{CaO} \cdot \text{Al}_2\text{O}_3 \cdot \text{Fe}_2\text{O}_3$ , by this method and it is hoped to attempt this shortly.

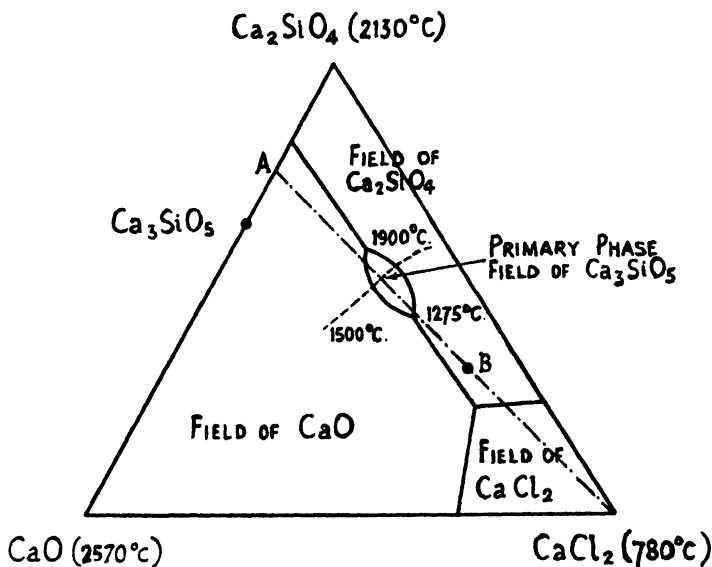


FIG. 6.—System  $\text{CaO}-2\text{CaO} \cdot \text{SiO}_2-\text{CaCl}_2$ .

**Crystallization from the Glass.**—Most studies of crystallization from the glassy state<sup>9,10</sup> have been carried out on commercial glass compositions. In such cases it has been assumed, and sometimes confirmed experimentally, that the phase crystallizing is that to be expected from the relevant phase equilibrium diagram. A number of observations, for which there is as yet no connected theory, indicates that this is not always the case.

In the crystallization of high alumina cement melts a large amount of a phase which is known as “unstable  $5\text{CaO} \cdot 3\text{Al}_2\text{O}_3$ ”<sup>\*</sup> (Fig. 7) often appears. Rankin and Wright found that both  $5\text{CaO} \cdot 3\text{Al}_2\text{O}_3$  and  $3\text{CaO} \cdot 5\text{Al}_2\text{O}_3$  occurred

<sup>8</sup> Nurse, unpublished data.

<sup>9</sup> Morey, *Properties of Glass* (Rheinhold Pub. Corp., 1938).

<sup>10</sup> Morey, *Trans. Faraday Soc.*, 1941, **37**, 209.

<sup>\*</sup> There seems to be little doubt that the stable phase identified by Rankin and Wright in the system  $\text{CaO}-\text{Al}_2\text{O}_3$  is  $12\text{CaO} \cdot 7\text{Al}_2\text{O}_3$  and not  $5\text{CaO} \cdot 3\text{Al}_2\text{O}_3$ . However, for the sake of clarity, the older designation will be used.

in unstable forms under certain conditions of cooling during their studies in the system  $\text{CaO}-\text{Al}_2\text{O}_3$ . Sundius<sup>11</sup> separated a mineral from high alumina cement clinker which corresponded closely in optical properties to Rankin and Wright's unstable  $5\text{CaO}\cdot 3\text{Al}_2\text{O}_3$ ; the separation was not complete and his analysis showed that the mineral might be  $3\text{CaO}\cdot 2\text{Al}_2\text{O}_3$ . Recent experiments at the Building Research Station indicate a composition higher in lime as being more likely, but still not conforming in composition to any compound known in the system  $\text{CaO}-\text{Al}_2\text{O}_3$ . Dyckerhof<sup>12</sup> obtained "unstable  $5\text{CaO}\cdot \text{Al}_2\text{O}_3$ " by annealing glass of that composition at about  $1000^\circ\text{C}$ . These experiments have been repeated at the Building Research Station, but owing to the dendritic nature of the crystals (Fig. 8) it was not possible to determine whether any glass remained. It was found, however, that the "unstable" compound was formed only below  $1020^\circ$ . The crystals in high alumina cement clinker are so well developed that it is difficult to believe that they are derived from devitrification of a glass; they frequently occur in association with the stable form.

A possible explanation is that a low-temperature stable form of  $5\text{CaO}\cdot 3\text{Al}_2\text{O}_3$  exists, or that at low temperatures  $5\text{CaO}\cdot 3\text{Al}_2\text{O}_3$  decomposes into  $\text{CaO}\cdot \text{Al}_2\text{O}_3$  and  $2\text{CaO}\cdot \text{Al}_2\text{O}_3$ , but there is little evidence to support this since crystalline  $5\text{CaO}\cdot 3\text{Al}_2\text{O}_3$  cannot be converted to the unstable form by annealing at any temperature. Furthermore the new species should crystallize from the melt in any polycrystalline system in which the liquidus temperature fell below the decomposition or inversion temperature of the normal form of  $5\text{CaO}\cdot 3\text{Al}_2\text{O}_3$ . Liquid temperatures in the system  $5\text{CaO}\cdot 3\text{Al}_2\text{O}_3-\text{Na}_2\text{O}\cdot \text{WO}_3$  fall below  $1020^\circ$ , but the primary  $5\text{CaO}\cdot 3\text{Al}_2\text{O}_3$  still occurs in the stable form.<sup>8</sup> There is some solid solution, however, so there is a possibility that the inversion (decomposition) temperature has been lowered.

If these aluminates prove to be truly metastable phases, this phenomenon may be of some interest in connection with recent studies of glass structures. Lukesh<sup>13</sup> has suggested that "structure phases" are formed in glass approximating, in the case of silicate glasses, to mica amphibole and pyroxene Si/O ratios, and having no relation to the composition of the phases formed on crystallization under equilibrium conditions. It is of interest also to note here that the glass of  $5\text{CaO}\cdot 3\text{Al}_2\text{O}_3$  composition has unusual properties showing a refractive index of 1.66 above that of stable  $5\text{CaO}\cdot 3\text{Al}_2\text{O}_3$  crystals (1.61) but below that of the unstable crystals (1.69).

The effect is not confined to the system  $\text{CaO}-\text{Al}_2\text{O}_3$ . Brownmiller<sup>14</sup> was the first to draw attention to the formation of metastable phases from glasses of compositions likely to be found in quickly cooled Portland cement clinker. Here again it appears possible that a corresponding mineral is sometimes found in thin sections of Portland cement clinker as a prismatic interstitial compound which according to Bogue<sup>15</sup> is unstable and formed only under unusual cooling conditions.

Bowen, Schairer and Posnjak<sup>16</sup> report an interesting observation on wollastonite. The inversion from high-temperature pseudo-wollastonite to low-temperature wollastonite is so sluggish that, once formed, it is almost impossible to convert it to the low-temperature form. The latter is, therefore, normally prepared by annealing glass at below  $1150^\circ\text{C}$  (the inversion temperature). These authors found that on annealing the powdered glass pseudo-wollastonite was obtained, whereas annealing a lump of glass

<sup>11</sup> Sundius, *Symposium on Chemistry of Cement* (Stockholm, 1938), p. 393.

<sup>12</sup> Dyckerhof, *Zement*, 1924, **13** (34), 400.

<sup>13</sup> Lukesh, *Science*, 1946, **104**, 199; *Amer. Miner.*, 1948, **33**, 76.

<sup>14</sup> Brownmiller, *Amer. J. Sci.*, 1938, **35**, 241.

<sup>15</sup> Bogue, *The Chemistry of Portland Cement* (Rheinhold Publishing Co., 1947), p. 132.

<sup>16</sup> Bowen, Schairer and Posnjak, *Amer. J. Sci.*, 1933, **26**, 207.

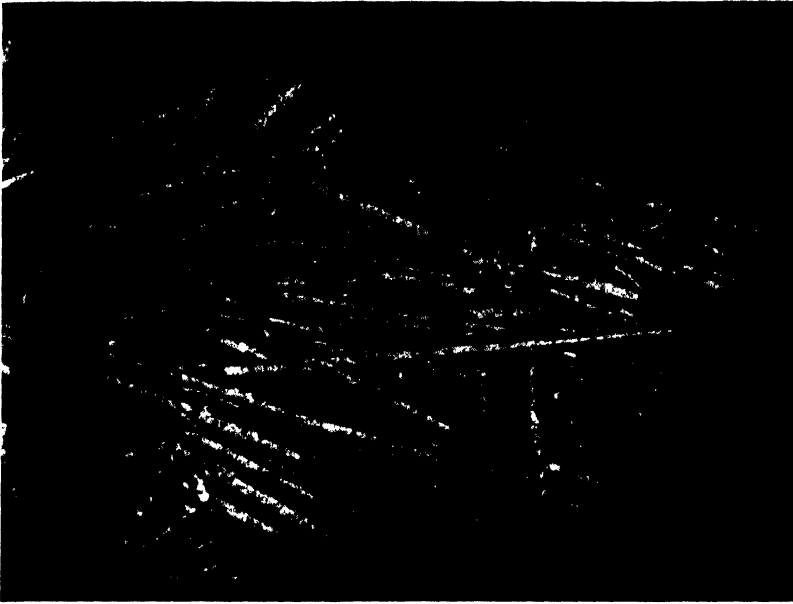


FIG. 7.—Unstable  $5\text{CaO} \cdot 3\text{Al}_2\text{O}_3$  in high-alumina cement clinker. ( $\times 50$ )



FIG. 8.—Annealed glass of composition  $5\text{CaO} \cdot 3\text{Al}_2\text{O}_3$  (crossed polars). ( $\times 250$ )





FIG. 9.—Section of twinned  $\beta$   $2\text{CaO} \cdot \text{SiO}_2$  crystal (crossed polars). ( $\times 50$ )

yielded the expected low-temperature wollastonite. These experiments when repeated by the present authors yielded the low-temperature form in both cases. The effect observed by Bowen is probably connected with the incomplete removal of pseudo-wollastonite nuclei from the glass, but, whatever the explanation for the beginning of crystallization, there seems to be no doubt that, once begun, the crystallization of pseudo-wollastonite continues at temperatures well below the inversion temperature.

The practical problem in building material manufacture is frequently the inverse of that considered here; namely, a study of the glassy condition and its influence in the properties of the product. The effect of glass formation in Portland cement clinker has been discussed by Parker.<sup>17</sup> In high alumina cement a low strength is often associated with excessive glass formation. On the other hand in granulating blast-furnace slag for cement production the maximum conversion to the glassy form is desired and this places an upper limit<sup>18</sup> on the lime content of slags used for this purpose, since otherwise crystallization cannot be inhibited by rapid cooling.

**Recrystallization in the Solid State.**—Growth of new crystals takes place during the decomposition or recombination of compounds to form new species on heating, by the decomposition on cooling of compounds which are stable only at high temperatures, and by inversion of polymorphic forms of one mineral.

An example of the former is the formation of  $3\text{CaO} \cdot \text{SiO}_2$  on heating a mixture of  $\text{CaCO}_3$  and  $\text{SiO}_2$ . After the decomposition of the carbonate the orthosilicate  $2\text{CaO} \cdot \text{SiO}_2$  is first formed, even if the temperature is above  $1275^\circ \text{C}$ , below which  $2\text{CaO} \cdot \text{SiO}_2$  and  $\text{CaO}$  are the stable phases. Tricalcium silicate is formed only after prolonged heating; an atmosphere of steam accelerates the combination. Although crystals of  $3\text{CaO} \cdot \text{SiO}_2$  formed in this way are only a few microns in size, good crystal faces are developed.

The same compound may be used as an example of the second type of reaction. Below  $1275^\circ \text{C}$  it decomposes in the solid state into  $\text{CaO} + 2\text{CaO} \cdot \text{SiO}_2$ . The onset of the reaction is seen as a development of turbidity within the tricalcium silicate grain which is resolved on further heating into birefringent specks of  $2\text{CaO} \cdot \text{SiO}_2$  and minute, rounded  $\text{CaO}$  crystals. The reaction takes several hundred hours to complete at  $1250^\circ \text{C}$  and even after this time the reaction products are imperfectly crystallized.

As might be expected from the high temperature at which they are formed, cement and slag minerals frequently show polymorphism. A new form of  $3\text{CaO} \cdot \text{SiO}_2$  has been found by Bernal as a result of X-ray examination of single crystals prepared by the technique developed by Nurse.<sup>7</sup> The crystallographic relations between the various forms of  $2\text{CaO} \cdot \text{SiO}_2$  on inversion have been discussed by Tilley<sup>19</sup> who has also discovered a new high-temperature form. Twinning in such compounds has been related to the polymorphism<sup>20</sup> and Parker and Ryder<sup>21</sup> conclude as a result of an empirical correlation of microscopic structure with dusting of blast-furnace slags that twinning is characteristic of those forms of  $2\text{CaO} \cdot \text{SiO}_2$  which are likely eventually to invert to the  $\gamma$  form. Twinning may also presumably arise as a result of stresses imposed by the cooling conditions, as well as from inversion. The twinned crystal of  $2\text{CaO} \cdot \text{SiO}_2$  shown in Fig. 9 was grown from melt entirely in the temperature range at which the  $\beta$  form

<sup>17</sup> Parker, *J. Soc. Chem. Ind.*, 1939, **38**, 203.

<sup>18</sup> Parker and Nurse, *Granulated blastfurnace slag for cement manufacture*. Building Research Technical Paper (H.M. Stationery Office) (in press).

<sup>19</sup> Tilley, *Miner. Mag.*, 1948, **28**, 255.

<sup>20</sup> Insley, Flint, Newman and Swenson, *J. Res. Nat. Bur. Stand.*, 1938, **21**, 355.

<sup>21</sup> Parker and Ryder, *J. Iron Steel Inst.*, 1942, **2**, 21P.

is stable, but there is no evidence to show whether the twinning existed before cooling the crystal to room temperature.

During the inversion of  $\beta$  to  $\gamma$   $2\text{CaO} \cdot \text{SiO}_2$ , an intermediate condition has been observed when the optical properties are partly those of one and partly of the other phase.<sup>21</sup> Such "metaphases" have been reported for a number of solid phase reactions. Eitel<sup>22</sup> has shown by means of electron microscopy and electron diffraction that, at any rate in a number of cases, there is no true intermediate phase, but that nuclei of the new phases form at definite points in the lattice of the decomposing phase, giving rise to anomalous optical properties.

**Crystallization in Aqueous Systems.**—The crystallization of solids in aqueous solutions is an important factor in the cementing of materials and under certain conditions in causing disruption.

The setting of plaster can be regarded as one of the simplest cases of growth of crystals which interlock and cement into a solid mass. Though the reaction



is accompanied by a decrease in volume of over 7 %, a mass of plaster expands on setting, leaving voids in its interior. The magnitude of the expansion, which is influenced by various factors and particularly by the presence of small amounts of other agents, usually falls within 0.1 to 1.0 % (linear). There is an initial stage while the mass is still very plastic in which a small contraction appears, but this is soon superseded by the expansion which runs roughly parallel to the rate of hydration. It is generally held that in the initial stage before the plaster acquires rigidity the crystals are free to move without restraint, but that as soon as a rigid structure is formed, unidirectional growth under conditions of restraint causes the observed expansion. Recent unpublished work by Andrews at the Building Research Station has shown that the degree of expansion is related to the crystal habit assumed by the gypsum. With a medium, such as water, in which the gypsum crystals grow in acicular form, a high expansion is normally found (Fig. 10), while in the presence of additions which lead to crystallization of the gypsum in less-elongated and broader forms the expansion is low (Fig. 11). The growth of crystals against a unidirectional stress seems to demand more study, since in the few recorded measurements the forces developed are generally small. An exception appears in the forces recorded by Correns and Steinborn<sup>23</sup> with crystals of potassium alum, but Schubnikow<sup>24</sup> for the same case found only very small forces. It is clear<sup>25</sup> on thermodynamical grounds that when a longitudinal compressive stress is applied to a crystal, without pressure on the surrounding solution, the solubility will be increased more at the stressed face than at the free faces. In the case of the setting of plaster there exists a considerable degree of supersaturation of the solution but no apparent relation between that degree and the form of the crystal growth.

Le Chatelier long ago advanced the general theory that cementing action occurred by crystal growth when a system of anhydrous constituents unstable in water reacted to produce a solution which was supersaturated with respect to the stable system of hydrated products. This applies to calcium sulphate plasters and to cements, though in the latter case the relative parts played by crystal growth and surface forces of gelatinous constituents has long been subject to controversy. An interesting example of cementing

<sup>21</sup> Eitel, *Preussische Akad. Wiss.*, 1943. *Math. Naturw.*, Klasse No. 5, Berlin, 1944.

<sup>22</sup> Correns and Steinborn, *Z. Krist.*, 1939, **101**, 117.

<sup>23</sup> Schubnikow, *Z. Krist.*, 1934, **88**, 466.

<sup>24</sup> Goranson, *J. Chem. Physics*, 1940, **8**, 323.

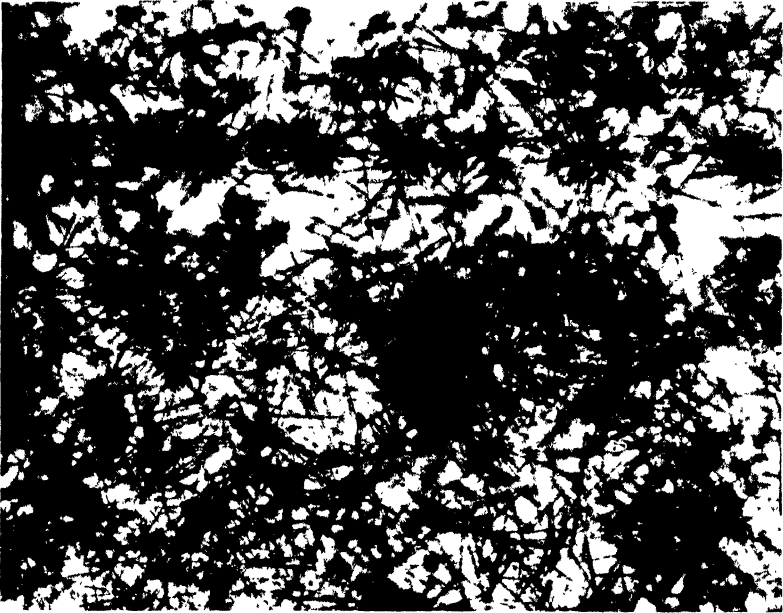


FIG. 10. —Elongated gypsum crystals grown in water. ( $\times 250$ )



FIG. 11.—Stubby modification of gypsum habit by sodium citrate. ( $\times 250$ )



action resulting from the transformation of a monotropic anhydrous compound to its stable form has been cited by Ilchenko and Lafuma.<sup>26</sup> The solubility of aragonite in water is slightly greater than that of calcite and a finely ground powder of the former was found to set very slowly with water owing to the growth of calcite crystals.

The conversion of one crystalline compound to another can also have destructive effects. The hydration of high alumina cement at normal temperatures leads to the formation of  $2\text{CaO} \cdot \text{Al}_2\text{O}_3 \cdot 8\text{H}_2\text{O}$  and gelatinous alumina. The hydrated calcium aluminate, which is formed as pseudo-hexagonal plate and needle crystals, is metastable with respect to the cubic compound  $3\text{CaO} \cdot \text{Al}_2\text{O}_3 \cdot 6\text{H}_2\text{O}$ , but at  $18^\circ$  it remains stable indefinitely. At higher temperatures ( $35^\circ - 50^\circ$ ) the inversion occurs within weeks, with liberation of hydrated alumina. Though accompanied by an increase in density and reduction in solid volume the change causes a loss of some 70–80 % of the strength of the set mass.<sup>27</sup> The initial strength development at  $45^\circ$  is also lower than at  $18^\circ$  indicating the poorer binding action of cubic crystals compared with more elongated forms. A similar effect has been found by Andrews with gypsum plasters where the higher strengths are associated with the more elongated forms of the gypsum crystals.

As another example of crystal transformation causing disruption, there may be cited the action of calcium sulphate solutions (or other soluble sulphates) on the hydrated alumina compounds present in set Portland cement. The compound  $4\text{CaO} \cdot \text{Al}_2\text{O}_3 \cdot 13\text{H}_2\text{O}$ , or certain solid solutions which it forms, reacts with calcium sulphate to form  $3\text{CaO} \cdot \text{Al}_2\text{O}_3 \cdot 3\text{CaSO}_4 \cdot 32\text{H}_2\text{O}$ . The former compound occurs as hexagonal plates and the latter as elongated hexagonal needles. The increase in solid volume which results is accommodated not by growth into existing pore spaces but by an outward thrust causing disruption of the solid mass. Lafuma<sup>28</sup> has suggested that the expansion is due to the low solubility of the compounds involved and direct growth from the original hydrated calcium aluminate crystals *in situ* rather than by passage into and growth from solution. An analogy may be drawn with the high pressures that can be created within a porous material containing a saturated solution of sodium sulphate and the anhydrous salt when the temperature is reduced below the transition point of the hydrated decahydrate.<sup>29</sup>

These various examples add emphasis to the comment made earlier on the need for more study of the growth of crystals under stress and reinforce the conclusion drawn by Wells<sup>6</sup> that more systematic work is needed on the factors which determine the relative rate of growth of different crystal faces.

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<sup>26</sup> Ilchenko and Lafuma, *Chim. et Ind.*, 1937, **38**, 438.

<sup>27</sup> Lea, *J. Soc. Chem. Ind.*, 1940, **59**, 18.

<sup>28</sup> Lafuma, *Rev. Mat. Const.*, 1929, **243**, 1929; 1930, **244**, 4.

<sup>29</sup> Bonnell and Nottage, *J. Soc. Chem. Ind.*, 1939, **58**, 10.

## THE GROWTH OF PERICLASE CRYSTALS AND ITS IMPORTANCE IN BASIC REFRACTORIES

BY E. B. COLEGRAVE, H. M. RICHARDSON AND G. R. RIGBY

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Although crystal growth is regarded as an important factor influencing the mechanical properties of ceramic bodies, there appear to be few, if any, investigations which have been made specifically to study this point. It has been suggested that a high mechanical strength in certain porcelain bodies is due to the growth of mullite crystals which can interlock owing to their acicular habit. In general, however, crystallization from a glass phase is accompanied by a deterioration in mechanical properties, and the larger the individual crystals, the more marked is the reduction in strength. This latter point is generally appreciated in the fabrication of high-temperature oxide materials by the process of sintering, the problem being to reduce the pore spaces while avoiding marked crystal growth. There is one refractory material, however, magnesia, which is preheated before use in order to encourage large crystals to develop. Commercial "magnesite" when made into bricks or blocks finds important industrial applications in the linings of basic steel furnaces, copper-refining furnaces, rotary cement kilns and metal mixers for pig iron. It is obtained by calcining either the natural carbonate rock or magnesium hydroxide obtained by decomposing the magnesium salts present in sea water. During this calcination the carbonate or hydroxide is decomposed to the oxide at temperatures below 800° C, but this so-called caustic magnesia is unsuitable for refractory uses, as bricks made from the material would possess excessive volume-shrinkage on being subjected to steel-making temperatures; further the bricks would hydrate fairly rapidly on exposure to the atmosphere which would cause them to crumble. The magnesia of the refractories industry has therefore to be calcined to a temperature of around, or above, 1600° C before being pressed into bricks, this high-temperature calcination being referred to as dead-burning. It has been known for many years that the dead-burning obviated excessive shrinkage and hydration tendency, the process generally being accompanied by a marked increase in specific gravity as determined by the conventional density bottle method. This increase in specific gravity between calcining at 1300° C and 1600° C was at one time thought to be due to the oxide altering its crystalline form, but X-ray analysis has subsequently shown that this is not the case, the effect of increasing the calcination temperature being to increase the size of the individual periclase crystals, the lattice remaining unchanged. Nevertheless, the specific gravity determination is often used as a criterion of the degree of dead-burning which the material has received. It has been known for many years that impure magnesite rock is more readily dead-burnt than the purer carbonates. The magnesite rocks from Styria are of the breunnerite type and contain from 5 % to 30 % of ferrous carbonate in solid solution with the magnesite. These sources supply an excellent dead-burnt oxide, whereas the purer Grecian magnesites are more difficult to dead-burn and the resulting oxide may still possess a residual shrinkage. The effect of impurities on the rate of crystal growth of the periclase crystals is a problem having practical applications, and when Germany, before the





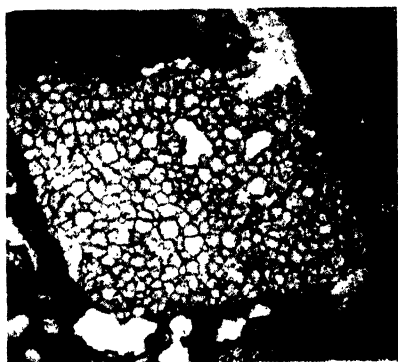


FIG. 1.—Rounded periclase crystals in a magnesia grain. ( $\times 40$ )



FIG. 2.—Periclase crystals showing the disappearance of boundaries and the development of cleavage. ( $\times 40$ )

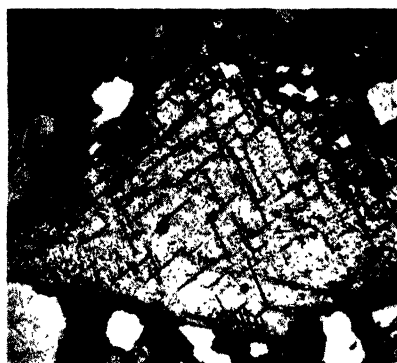


FIG. 3.—The small crystals have lost their identity and cleavage has developed in two directions. ( $\times 40$ )



FIG. 4.—Periclase crystals from the cooler face of a brick after use ( $\times 66\frac{2}{3}$ )



FIG. 5.—Periclase crystals from the working face of a brick after use. ( $\times 66\frac{2}{3}$ )

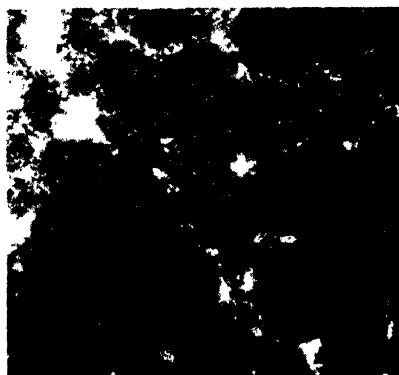


FIG. 7.—Periclase crystals with numerous small inclusions of magnesioferrite. ( $\times 106$ )

annexation of Austria in 1936, banned the import of Austrian magnesite, patents were taken out to encourage crystal growth of the purer magnesites by additions of calcium and magnesium ferrite.

The sizes of individual periclase crystals in magnesite refractories can be readily measured by examination of a thin-section under the microscope. Chesters, Clarke and Lyon<sup>1</sup> found that the average diameter of periclase crystals measured by this means agreed well with the size obtained from a study of X-ray back-reflection photographs. These authors found that various commercial magnesite bricks contained crystals of an average diameter 0.6 to 0.8 mm. Later Jay and Chesters,<sup>2</sup> examining a range of magnesite bricks, found a wide variation in crystal size, although in any given brick the size was fairly uniform. In such bricks the periclase crystals are usually rounded as shown in Fig. 1 but after receiving more severe heat treatment one or more cleavages are developed, the crystals at the same time increasing in size (Fig. 2). Finally, the crystal may show well-defined cleavage cracks in two directions as shown in Fig. 3.

The significance of the crystal growth and development of cleavages which usually occurs at the face of a magnesite brick exposed to severe temperatures over long periods as well as to the action of slags and vapours is not known with certainty. Jay and Chesters<sup>2</sup> have suggested that this crystal growth may lower the resistance of the material to withstand sudden temperature changes without cracking although the development of cleavages may have the reverse effect. Fig. 4 and 5 illustrate the growth which can occur in the individual periclase crystals composing a magnesite brick during service; the periclase crystals in Fig. 4 taken from the colder face of the brick after service are probably of the same order of magnitude as those in the brick before use, but it can readily be seen from Fig. 5 that the periclase crystals at the hot face have increased appreciably in size and have developed a distinct cleavage.

#### The Effect of Temperature and Impurities on Crystal Growth.—

Measurements of the variation in crystal growth of periclase crystals with calcining temperature have been made by several investigators. Letort and Halm<sup>3</sup> selected a product from sea water containing 2.5 % CaO, 0.5 % SiO<sub>2</sub>, 0.5 % Fe<sub>2</sub>O<sub>3</sub> and 0.10 % Al<sub>2</sub>O<sub>3</sub> and measured the crystal growth by use of the microscope. A general increase in crystal size was noted as the calcination temperature was raised from 1600° to 1800° C. Growth also occurred on increasing the time of heat treatment, as on maintaining a temperature of 1800° C for one hour the crystals increased in length from 10–15  $\mu$  to 35–40  $\mu$ . The effect of impurities in promoting crystal growth has also received attention, Letort and Halm<sup>3</sup> found that additions of 5 % Fe<sub>2</sub>O<sub>3</sub> and 3 % SiO<sub>2</sub> to the sea-water magnesia resulted in periclase crystals 60–70  $\mu$  and 50–60  $\mu$  in length respectively on heating for one hour at 1600° C, whereas if no additions were added the crystal size remained at 25–30  $\mu$ . These investigators also observed that a reducing atmosphere promoted crystal growth. The importance of kiln atmosphere on the structure of magnesite refractories was also emphasized by Krause and Ksinsik<sup>4</sup> although they consider that, in the dead-burning process, atmosphere is only of secondary importance. Jay<sup>5</sup> has used X-ray methods to estimate crystal size, and the graph Fig. 6 shows the variation in crystal size with temperature for three different magnesites. Sample 1 had a very

<sup>1</sup> Chesters, Clarke, and Lyon, *Trans. Brit. Ceram. Soc.*, 1935, **34**, 243.

<sup>2</sup> Jay and Chesters, *Trans. Brit. Ceram. Soc.*, 1938, **37**, 218.

<sup>3</sup> Letort and Halm, *Chim. et Ind.*, 1947, **58**, 537.

<sup>4</sup> Krause and Ksinsik, *Feuerfest*, 1932, **8**, 6.

<sup>5</sup> Jay, *J. Sci. Instr.*, 1941, **18**, 81.

low content of impurities while sample 3, on analysis, contained 5 % of oxides other than magnesia.

Tentative theories have been suggested from time to time to try and explain the mechanism by which certain impurities foster crystal growth. Both Letort<sup>5</sup> and Konopicky<sup>6</sup> consider that ferric oxide is combined as magnesioferrite which is soluble in the periclase crystals at high temperatures, although this may be precipitated on cooling. When magnesite refractories are exposed for considerable periods to ferruginous slags, the surface of the brick may consist of magnesioferrite with magnetite in solution, but immediately behind this layer it is common to find large periclase crystals containing minute inclusions of magnesioferrite, which have presumably been deposited from solution on cooling (Fig. 7). Periclase and magnesioferrite both crystallize in the cubic system though the cell size of MgO is only half that of the spinel. Tanaka<sup>7</sup> found that titanium dioxide additions

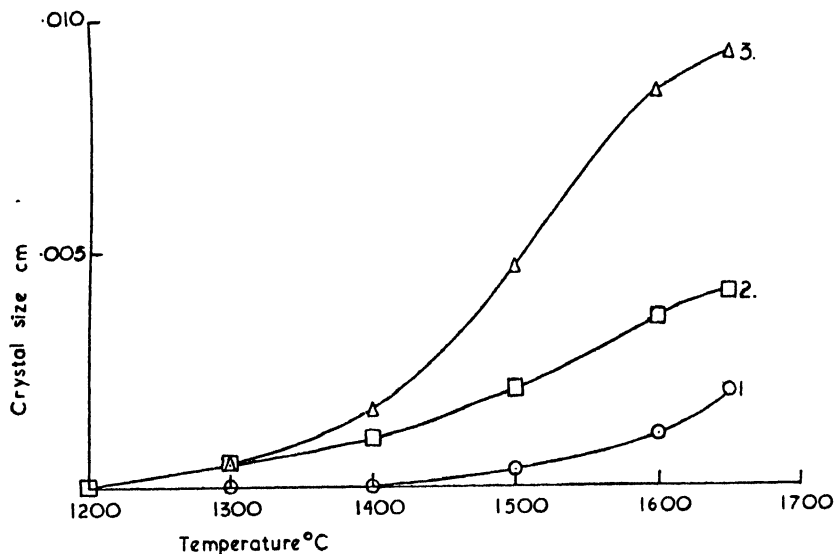


FIG. 6.

also facilitated the sintering of magnesia, and he attributed this to the formation of magnesium orthotitanate which also has a spinel structure. Theories based on solution effects promoting crystal growth, however, cannot be used to explain the mineralizing effect of silica and lime, as it is unlikely that these oxides form minerals which are soluble to any extent in the periclase crystals, and Krause and Ksinsk<sup>4</sup> have stated that the effect of iron oxide on the dead-burning process is less than that of lime or silica. It is, of course, possible that at high temperatures, iron oxide is in solution in the periclase crystals as FeO since FeO and MgO are completely miscible in all proportions in the solid state, and that the marked defect structure of FeO might promote crystal growth. It has been shown

<sup>6</sup> Konopicky, *Ber. dt. Keram. Ges.*, 1937, **18**, 97.

<sup>7</sup> Tanaka, *J. Soc. Chem. Ind., Japan*, B, 1939, **42**, 202.

that at high temperatures magnesioferrite is partly decomposed owing to loss of oxygen. The effect of a reducing atmosphere in enhancing crystal growth could be explained by any theory which postulated the partial dissociation of magnesioferrite to  $\text{FeO}$  followed by its reformation on cooling, and it is probable that measurements of oxygen pressures in the  $\text{MgO-FeO-Fe}_2\text{O}_3$  system would provide useful confirmatory data.

Recent data obtained by the authors on the effect of growth of the periclase crystals with (a) temperature and (b) additions of ferric oxide are given graphically in Fig. 8.

The magnesite was a natural rock which on calcination contained only 3.56 %  $\text{SiO}_2$ , 0.40 %  $\text{Fe}_2\text{O}_3$ , 0.74 %  $\text{Al}_2\text{O}_3$ , 0.66 %  $\text{CaO}$  and 2.12 % alkali metal oxides as impurities. The ferric oxide additions were introduced as

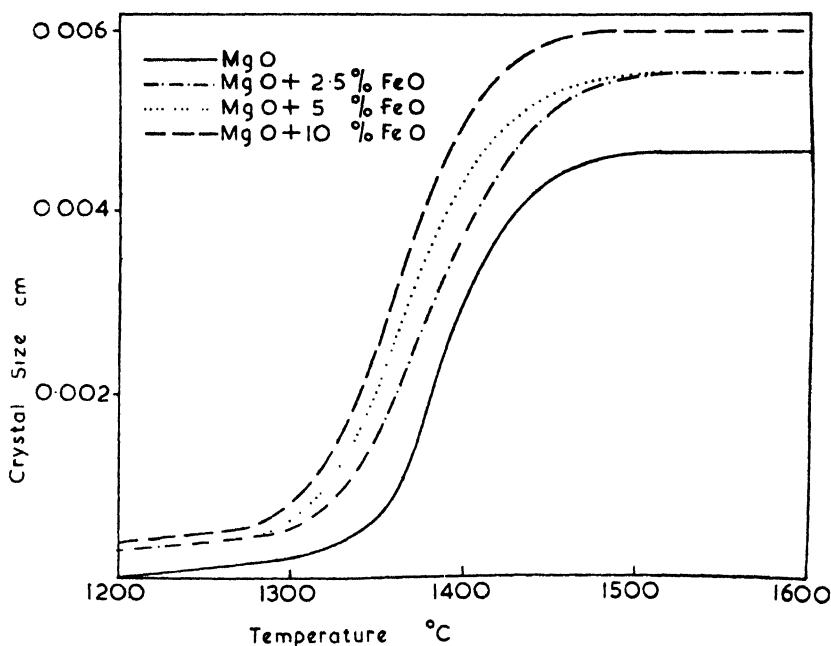


FIG. 8.

ferrous oxalate which was dry-ground with the magnesite rock after a preliminary calcination at  $800^\circ\text{C}$ . The results showed that a large increase in crystal size was observed as the calcining temperature was raised from  $1350^\circ\text{C}$  to  $1450^\circ\text{C}$ .

**The Effect of the Growth of Periclase Crystals on (1) the Volume Shrinkage, (2) the True Specific Gravity and (3) the Hydration Tendency.**—The authors have investigated the volume shrinkage undergone by magnesia on heating at various temperatures up to  $1600^\circ\text{C}$  by measuring the diameters of standard cylinders made by compressing the powdered magnesia calcined to  $800^\circ\text{C}$ . The effect of additions of  $\text{Fe}_2\text{O}_3$  up to a maximum amount of 5 % was also studied and the results given in graphical

form in Fig. 9. It may be observed that maximum shrinkage occurred over the range 1350° to 1450° C, but the specimens were still shrinking at 1600° C.

The true specific gravities as determined by displacement methods of various commercial dead-burnt magnesites may vary from 3.56 to 3.65. Jay and Chesters<sup>2</sup> have emphasized that the crystal size, and therefore the degree of dead-burning of the magnesite, bore no simple relation to the specific gravity unless a correction was first made to allow for the iron oxide content of the brick. When using specific gravity values as a criterion of dead-burning it is usual to assume an increase of 0.01 in the specific gravity for each per cent. of  $\text{Fe}_2\text{O}_3$  in the magnesite analysis. According to calculations made from X-ray photographs the theoretical density of

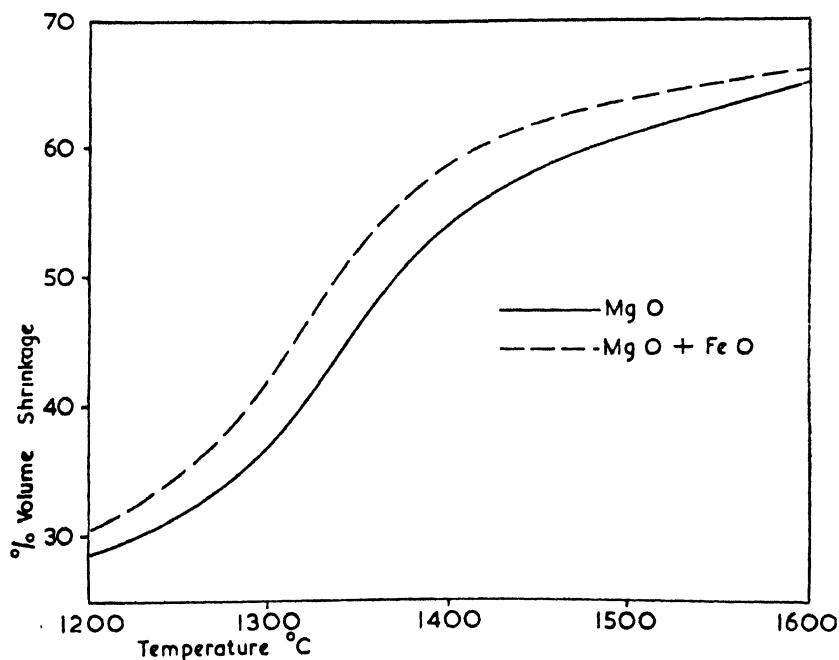


FIG. 9.

pure magnesia is 3.58 and this does not alter with the temperature of calcination. In the above samples, the variation in specific gravity with the temperature of calcination as determined by density bottle measurements ranged from 3.490, with no addition on calcining to 1200° C, to 3.607 with the addition of 5 %  $\text{Fe}_2\text{O}_3$  after a calcination to 1600° C. These data can only be reconciled with the X-ray results by assuming that at low temperatures of calcination the magnesia contains micro-pores to which the penetrating liquid used in the density bottle cannot gain access. Micro-pores enable lightly calcined magnesia powder to be used as a thermal insulator, and their disappearance on increasing the calcining temperature is accompanied by an overall volume shrinkage.

The relationship between calcination temperature and hydration tendency

is given by the curves in Fig. 10. The hydration tendency is measured by exposing the finely powdered magnesia to steam at  $100^{\circ}\text{C}$  for 5 hr., drying finally to  $110^{\circ}\text{C}$  and determining the loss in weight on igniting the sample. It will be observed that a sharp decrease in the hydration tendency occurred as the calcination temperature was raised from  $1300^{\circ}$  to  $1400^{\circ}\text{C}$ .

**Conclusions.**—It will readily be appreciated that previous investigations on the changes taking place during the dead-burning of magnesia have been made mainly with the practical object of obtaining a better product at lower cost. Among the more theoretical aspects which require further

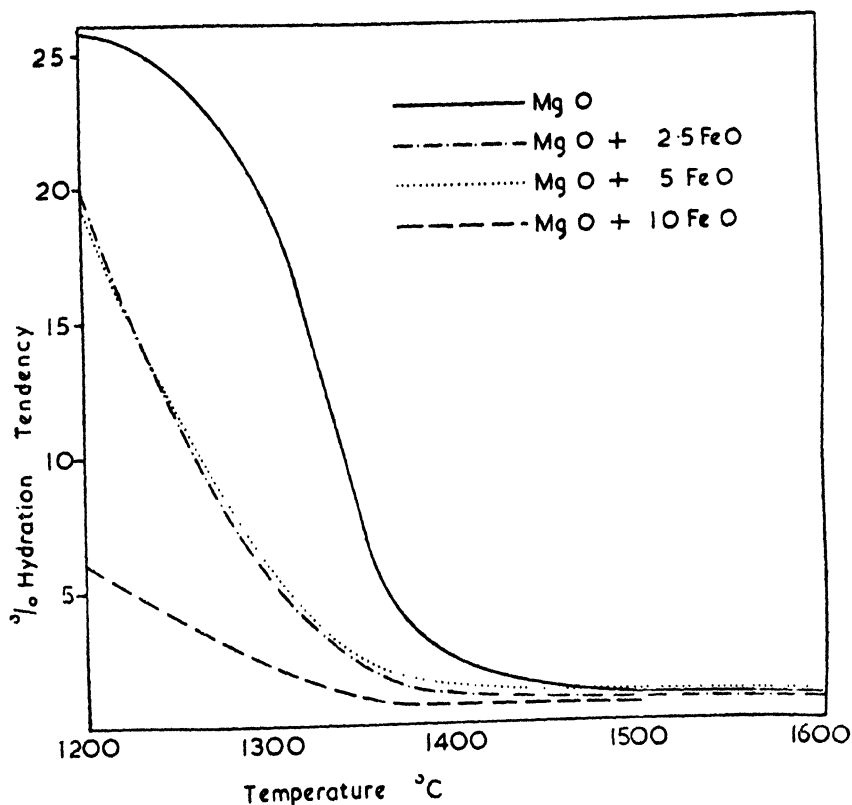


FIG. 10.

elucidation are the mechanism by which crystal growth occurs and is promoted by certain mineralizers, and the significance to be attached to the development of cleavage planes on subjecting periclase crystals to high temperatures for long periods.

The authors are grateful to the Director of the British Ceramic Research Association, Dr. A. T. Green, O.B.E., for permission to publish this paper.

*British Ceramic Research Association,  
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## GENERAL DISCUSSION

**Mr. Y. Haven** (*Findhoven*) said: Several authors reported that impurities may be useful in making good crystals. We, too, have made several good, single LiF crystals from a melt to which  $\text{MgF}_2$  (up to a few 0.1 %) was added, in contradistinction to Prof. Stockbarger's experiences who stated that the LiF should be of the utmost purity.

The influence of the impurities may be discussed in the following way. On the one hand, for lattice defects in ionic crystals (in most cases, vacant lattice sites) the law of mass action should be approximately valid, i.e., the product of the concentrations of vacant  $\text{Li}^+$  sites and vacant  $\text{F}'$  sites in LiF should be a constant so that one may decrease the number of vacant  $\text{F}'$  sites by increasing the number of vacant  $\text{Li}^+$  sites (e.g., by substituting  $2\text{Li}^+$  ions for  $1\text{Mg}^{++} + 1$  vacant site).

On the other hand, one kind of ion may often take a dominant part in building up an ionic lattice, thus the negative ions often form the lattice framework, the positive ions having only a more or less supplementary function. This manifests itself by the fact that it is often far more difficult to replace the negative ions than to replace the positive ions.

Now defects in the lattice of the framework ions may be more harmful for growing good crystals than the defects of other lattice sites. Thus in LiF one has to prevent defects in the  $\text{F}^-$  lattice, which may be accomplished by adding  $\text{Mg}^{++}$  ions.  $\text{Mg}^{++}$  ions (i.e., vacant  $\text{Li}^+$  sites) may therefore have a healing effect on the  $\text{F}^-$  lattice in LiF. For the same reason, it is possible that one must not push the purification too far, for by removing the positive ion impurities one may introduce negative ion defects.

I would recommend the use of  $\text{NH}_4\text{F}$  in making LiF single crystals. The LiF is mixed with an excess of  $\text{NH}_4\text{F}$ . On heating, the  $\text{H}_2\text{O}$  is evaporated first and then the excess of  $\text{NH}_4\text{F}$ . In this way hydrolysis does not give any trouble, even when the crystal is in contact with air. I would also like to ask why Prof. Stockbarger prefers the crucible-lowering method to that of Kyropoulos, since the latter has the advantage of easy manipulation and inspection and the temperature gradient is such as to cause a good circulation in the melt, which may be important for the growth of the crystal.

**Dr. W. Ehrenberg and Mr. J. A. Franks** (*London*) said: We obtained good results in the preparation of single alkali halide crystals by using a modification of Stöber's technique. Our furnace has a high thermal inertia; the main heaters at the top and bottom consist of Kanthal strip and wire embedded in ceramic. No mains stabilization is used. The temperature and temperature gradient are measured with Pt-PtRh thermocouples permanently inserted in the melt; this appears to be the only way of obtaining information about the actual conditions.

Single crystals are almost invariably obtained, even with flat-bottomed crucibles, provided a positive temperature gradient (top at the higher temperature) is maintained throughout the crystallization. No crucible material was found to which the crystals do not adhere; therefore, in order to avoid strains being introduced, it is necessary to use very flimsy containers: our crucibles are made simply by bending a circular sheet of  $1/1000''$  Pt foil around a former resembling a cog-wheel; the crucibles look like fairy-cake tins about  $2\frac{1}{2}''$  diam. and  $1''$  high. This peculiar shape does not interfere with the growth of the crystal.

The crystals are partially orientated with respect to the flat bottom (100 axis parallel to bottom); an orientation with respect to the temperature gradient cannot be expected for alkali halides; in general, a careful study of the literature gave us no evidence that such an orientation has ever been observed. In all cases some Pt is dissolved in the melt and collects at the top. The bulk of this appears as a dirty irregular deposit on the top of the crystal, while the bottom half is pure; the top half appears cloudy under u.v. light. One KCl crystal grown in an Au crucible beautifully demonstrates the distribution of impurity (Au), the crystal being quite colourless near the bottom and turning deep purple towards the top, on which specks of a thin metallic gold deposit are visible.

We can observe no strains in crystals cleaved by tapping with a blunt tool. Strains appear, however, in crystals cleaved with a chisel.

**Mr. P. R. Rowland** (*London*) said : From what Dr. Ehrenberg has said, there appears to be no orientating effect due to the thermal gradient in the growth of alkali halides from the melt. This is, however, not always the case with metals. Prof. G. I. Finch informs me that in the case of zinc, which has a close-packed hexagonal structure, it is difficult to grow a single crystal unless there is only a small angle between the line of steepest descent of the temperature and the cleavage plane of the crystal. However, in the case of copper, which is cubic close-packed, there appears to be no such effect. We have grown many and have not been able to observe it (though the number is insufficient to justify a statistical analysis). Neither have we found any reference in the literature to such an orientating effect. There may be an exception to this in one of our experiments, where a thin strip was grown from the melt. The crystal formed had cube direction at an angle of  $18\frac{1}{2}^{\circ}$  to the surface plane of the strip. About  $1\frac{1}{2}$  cm. from the lower (first formed) end of the crystal a second one about  $1\frac{1}{2}$  mm. wide appeared approximately in the centre of the strip, which was 1 cm. wide. This second crystal had a cube direction at an angle of only  $8^{\circ}$  to the surface. The two shared a common cube direction which lay in the surface plane and was normal to the direction of growth. Is it possible that this second crystal appeared because there is a tendency to orientate with a cube direction parallel to the direction of growth? Since the strip was thin (0.5 mm.) an alternative is that surface energy effects may tend to orientate with a cube plane in the surface.

**Mr. P. R. Rowland** (*London*) (*communicated*) : Since making the above remarks, the author has discussed the matter with Dr. W. Willman and Prof. G. I. Finch and finds that he was under a slight misapprehension. Zinc crystals grow most quickly parallel to the cleavage plane, and hence a seed crystal orientated with this plane nearly parallel to the length of a rod-shaped melt will tend to outgrow others. There is thus no evidence that the thermal gradient has any effect in orientating the seed in the growth of either zinc or copper from the melt.

**Mr. T. A. Kletz** (*I.C.I., Billingham*) (*communicated*) : In their paper Dr. Menzies and Dr. Skinner state that the discovery of the transmission of silver chlor  $^{\circ}$  in the infra-red is probably to be ascribed to Dewar. Actually the discovery was made by Schulze-Sellack nearly 80 years ago,<sup>1</sup> later two of the early workers on infra-red spectroscopy, Rubens and Nichols, used silver chloride for the window of their radiometer, taking measurements <sup>2</sup> at wavelengths up to 24  $\mu$ .

**Mr. A. E. Robinson** (*R.N.S.S.*) said : My paper describes plant and apparatus in use for growing small supplies of single crystals for development purposes. The principles do not differ from those in use by a number of workers in this field. A similar ammonium dihydrogen phosphate plant was operated by the Ministry of Supply for the Admiralty before this unit was assembled.

In single crystal growing a main concern is the severely practical problem arising from what Dr. Holden has referred to as "maintaining a metastable system for long periods of time."

Of general interest, however, is the fact that the work affords opportunities for observing considerable numbers of crystals of a size and quality not usually met with, and points which may be missed in smaller crystals become obvious. The first is that rate of growth depends on quality. Under given conditions crystals free from obvious flaws do grow at a reasonably constant and uniform rate; seeds with inherent flaws which persist grow at higher rates and the most flawed crystals, but which still retain geometric identity, grow at the highest rate, which is three to four times as great as the slowest-growing sound crystals.

These are observations on crystals grown in solutions free from significant impurities. In general foreign substances in the solution fall into three groups: those without significant effect, e.g., 1 % or 2 % of sodium sulphate in lithium sulphate solutions; those with specific effect, and here it must be remembered

<sup>1</sup> Schulze-Sellack, *Pogg. Ann.*, 1870, **139**, 192.

<sup>2</sup> Rubens and Nichols, *Ann. Physik*, 1897, **60**, 418; and Baly, *Spectroscopy*, 3rd ed., vol. 1 (Longmans, 1924), p. 230.



the amounts required may be as small as 20 parts per million or less ; and those which appear to encourage the required sound growth, e.g., the small amounts of iron added to ammonium dihydrogen phosphate solution.

**Dr. E. W. Fell** (*Bradford*) said : Dr. Holden and Mr. Robinson describe a method of growing crystals by moving them through the surrounding solution. However, I should like to refer briefly to a mode of growth in melts when the melt is in motion. When a melt flows along a solid surface such as a mould wall, and under conditions whereby heat is rapidly conducted from the melt through the mould wall, crystallization proceeds from the surface and long crystals grow into the melt, and these crystals, of which the solidified layer near the mould wall is composed, are found to be inclined towards the direction from which the stream is coming. If there is no stream, the crystals grow approximately normally to the mould wall. Such growth is of interest in metallurgy.<sup>3</sup> It has been observed in steel ingots, lead and stearine. For steel ingots the inclination of the crystals to the normal to the mould is about 10°, for stearine about 14° and for aluminium containing 10 % magnesium (poured down one side of the mould) as much as 20°. The effect on the inclination of different velocities of the stream was not investigated, though there was probably a strong forced circulation of melt in the mould in the case of the aluminium alloy. It is reported that growing crystals of electro-deposited nickel are similarly inclined if the electrolyte is in motion. I have no information regarding crystallization from a solution in motion, but the method of growing crystals described by the authors suggests that a somewhat similar inclination as for melts may occur. It seems that the stream promotes nucleation and the removal of barriers to growth on that boundary of the growing crystal facing the oncoming stream of melt and hence the crystal grows more there, whereas on the other side of the crystal that is sheltered from the oncoming stream there is less nucleation and barrier removal. I should be glad to have the authors' views as to the cause of this observed inclination.

**Mr. A. E. Robinson** (*R.N.S.S.*) said : The phenomena reported by Dr. Fell<sup>4</sup> occur in crystallization from melts, whereas my paper refers to the somewhat different condition of growth from solutions.

The following points may, however, be of interest. In crystal growth in solution there is a tendency for increased growth on the face normal to the stream, and in practice the growing crystal is deliberately oriented so as to encourage growth in the required direction. In spontaneous nucleation, such as that reported on the cold mould wall, there appears to be a tendency for a particular face to adhere, and this is somewhat similar to my exhibit of extraneous growth on selected faces. The angles adopted by different materials are probably influenced by the morphology and habit of the crystal, the wetting of the mould wall and the fact that in a given crystal individual faces have their own degree of wetting by particular fluids.

**Dr. B. Raistrick** (*Birmingham*) said : When sodium trimetaphosphate crystallizes from a melt bubbles of what is believed to be moisture can be seen escaping from the crystal surface as growth proceeds ; the loss is weighable. We believe that this observation can be explained on chemical grounds, but would be interested to hear of any other cases of melts absorbing moisture which is then liberated on crystallization.

**Prof. R. M. Barrer** (*Aberdeen*) said : In my paper I have given a selection from the many factors which govern the growth of crystalline silicates. The discussion centres round methods of synthesis, crystal dimensions and some of the variables which control growth, including functions of mineralizers. A big literature has grown up in mineral chemistry, but in this country chemical aspects of mineral growth have failed to attract attention in the same way as some better-known phases of chemical research. Much exploratory work was done long ago—perhaps among the first published silicate syntheses one may name Schafheutl's preparation of quartz in 1845.<sup>5</sup> On the Continent there has

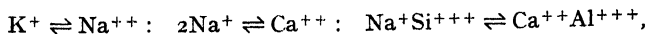
<sup>3</sup> Carlsson and Hultgren, *Jernkontorets Ann.*, 1936, 120, 577.

<sup>4</sup> This Discussion.

<sup>5</sup> *Munchner gelehrte Anzeigen*, 1845, 557.

been continued activity over a long time, and since the turn of the century this is also true of the United States. We owe to Morey and his colleagues in America many of the principal quantitative measurements using the hydrothermal technique.

Next to compounds of carbon those of silicon are among the most numerous. Silicates also comprise in bulk a large part of the lithosphere, but techniques for growing silicate crystals are unusual, often falling outside the range of ordinary chemical experience. The crystals are mainly of the "giant molecule" type, containing very large anionic networks, corresponding to chains, sheets and three-dimensional frameworks, and most usually grow from magmas of high viscosity at high temperatures. Many of the crystals transgress the law of constant proportions due to isomorphous replacements such as



and optical, X-ray and chemical data may all be required to establish identity of species. Chemists working in this field naturally depend upon mineralogy and geology in the first place to show the conditions which are likely to yield some at least of the various species.

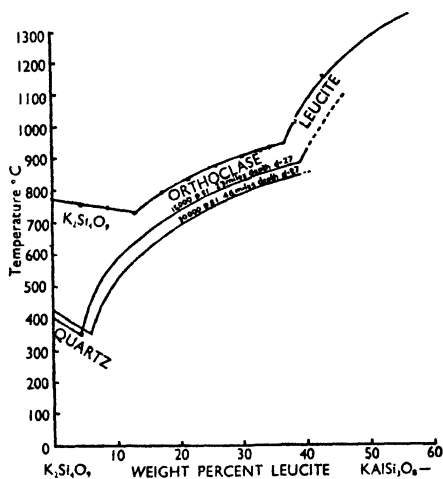


FIG. 1.

Nevertheless synthetic silicate chemistry will no doubt move in different directions and will reveal many species and types of behaviour not hitherto observed in nature. To some extent this is already true. Thus in my own experience, either by using standard methods or by developing new procedures, crystals have been grown apparently as yet not noted in nature. As examples of new syntheses by standard methods there are the growth by the hydrothermal technique of a new barium zeolite with chabazite-like sorptive properties and of several intermediate species containing the constituents  $BaCl_2$ ,  $BaBr_2$ ,  $KCl$  and  $KBr$  in solid solution throughout aluminosilicate frameworks. Dr. Taylor at my suggestion has by the same method successfully crystallized some thallos aluminosilicates, and Mr. White and also myself have grown a number of lithium aluminosilicate crystals which do not so far appear to have naturally occurring counterparts.

As an example of new methods one may mention the Clark and Steiger procedure for easy production of a number of ammonium minerals by ion interchange using  $NH_4Cl$  vapour. The  $NH_4^+$  ion is not found in natural aluminosilicates. I have been able to develop this procedure further in several cases by burning out the ammonium ions with  $O_2$  gas to form crystalline hydrogen chabazite and mordenite. In natural conditions hydrogen zeolites are never found.

Again by indirect methods one may easily obtain species which cannot be grown *directly* under the same conditions of pressure and temperature. Prof. Wyart reports that he has not been able to grow leucite directly by hydrothermal methods; however, I have made it easily by first growing analcite hydrothermally and then submitting this species to the cation interchange,  $\text{Na}^+ \text{H}_2\text{O} \rightleftharpoons \text{K}^+$ . In this preparation, reaction goes so easily that it is not necessary to work appreciably above  $200^\circ \text{C}$  whether in analcite synthesis or in ion interchange. Clearly synthetic methods are destined to extend and diversify knowledge given by the natural reactions of mineral chemistry.

With regard to mineralizers, perhaps water is the most universal, and in connection with its mode of action I wish to show an additional diagram containing results very recently published by Tuttle.<sup>6</sup> I noted that water may act not only by lowering the viscosity of melts, but also by lowering the crystallizing temperatures from the magma. This mechanism is especially important in growth of feldspathic crystals which somehow occurs very well at rather low temperatures from magmas which if anhydrous would be of astronomical viscosity. Fig. 1 shows the fusion curves of  $\text{K}_2\text{Si}_2\text{O}_6$  (or quartz), orthoclase and leucite in equilibrium with anhydrous and hydrous magmas. Curve 1 is for an anhydrous magma; curve 2 for a magma under a water pressure of 15,000 lb. in.<sup>-2</sup> (i.e., 2.3 miles deep); curve 3 for a magma under water pressure of 30,000 lb. in.<sup>-2</sup> (i.e., 4.6 miles deep). The fusion temperature of  $\text{KAlSi}_3\text{O}_8$  is considerably lowered as also is that of leucite. Water at these pressures actually eliminates growth of  $\text{K}_2\text{Si}_2\text{O}_6$ ; instead, quartz appears at a temperature  $300^\circ \text{C}$  lower.

**Prof. W. E. Garner** (*Bristol*) said: In order to account for the effect of mineralizers in the crystallization of quartz, it is possible that the mineralizer facilitates the crystallization at the repeatable step by increasing the mobility of the silica molecules over the quartz surface or in the adjacent liquid phase.

**Dr. G. R. Rigby** (*Stoke-on-Trent*) said: Dr. Barrer has mentioned that leucite has not yet been successfully synthesized by hydrothermal methods—no doubt Dr. Barrer knows that leucite can be synthesized readily by heating the requisite proportions of potash, alumina and silica and it is often found in used blast-furnace linings where firebricks have been exposed to potash vapour. This artificial leucite exhibits all the characteristic properties of the natural mineral, e.g., the polygonal form, low birefringence and cross-hatched twinning.

With regard to Dr. Van Praagh's paper I am surprised that he has identified the high-temperature form of cristobalite at room temperatures. Cristobalite is the stable modification of silica above  $1470^\circ \text{C}$ , but in practice it is often obtained by exposing quartz or fused silica to temperatures above  $870^\circ \text{C}$ . If, however, quartz is heated under molten sodium chloride, tridymite is obtained, thus illustrating the specific effect of mineralizers. The inversion of high to low cristobalite is accompanied by an increase in volume amounting to over 3.0 %, and this is often detrimental to ceramic materials containing the mineral. If the high-temperature form could be stabilized, thus inhibiting this inversion, it would mark a great advance in ceramic technology.

**Prof. R. M. Barrer** (*Aberdeen*) said: Questions have been asked about the growth of garnet and the functions of mineralizers, and the appearance of leucite in the glass-making furnace has been commented on.

Pyrolytic syntheses of leucite are very common, and it is unnecessary to attempt to summarize them. One is in no way surprised at its appearance during glass-making operations. What is still doubtful, however, is its growth by *direct* hydrothermal methods at low temperatures. Thus Prof. Wyart<sup>7</sup> has not succeeded in repeating Friedel's claims, and although I have carried out *direct* hydrothermal crystallizations of potassium aluminosilicate gels of varied compositions to give diverse species, leucite has not so far been noted among them, at least up to  $360^\circ \text{C}$ . On the other hand, by the *indirect* hydrothermal route, already referred to in my paper, leucite has been very easily made at temperatures of *ca.*  $200^\circ \text{C}$ .

<sup>6</sup> *Amer. J. Sci.*, 1948, **246**, 31.

<sup>7</sup> This Discussion.

A detailed mechanism cannot at present be given for the growth of garnet in metamorphic conditions. Nevertheless its appearance under high pressures is favoured by its large density according to thermodynamic principles. The symmetrical growth suggests a plastic flow or softening of neighbouring crystalline species of lower density also under the great pressure and in contact with the growing garnet nucleus or crystallite. At the surface of contact the chemical constituents of the other species are then reorganized under stress, so as to decrease the volume occupied and relieve the stress, by continuing the development of the garnet.

In discussing the action of mineralizers a number of speculations have been made. One should not, however, in devising special mechanisms, forget the quite normal aspects. These are that the mineralizer may lower the viscosity of the medium and so promote mixing and crystal growth; that it may alter the solubility and fusion temperatures of crystallizing species; and that it may form intermediate compounds. There is good evidence that examples of all these effects occur in various instances.

**Mr. R. W. Nurse** (*D.S.I.R., Watford*) said: Since our paper was written new information has come to hand concerning some of the examples cited. Sirota<sup>8</sup> has discussed the crystallization of metastable phases, particularly in binary metal alloys, using the Volmer-Stranski method for obtaining the work of formation of two- and three-dimensional nuclei. The examples given show that in general there are three temperature domains: a high-temperature region in which only the stable phase crystallizes, a low-temperature region in which only the metastable phase crystallizes, and an intermediate range in which the phase crystallizing depends on the kind of nuclei present. The theory gives a qualitative explanation of the behaviour of the unstable aluminates and also explains the continued growth of pseudo-wollastonite in the wollastonite field as observed by Bowen.

Trommel,<sup>9</sup> by means of X-ray studies in the high-temperature camera, finds that  $\beta_2\text{CaO} \cdot \text{SiO}_2$ , previously thought to be a high-temperature modification, is stable only at low temperatures. In the first cycle he obtains the inversion  $\gamma \rightarrow \alpha'$  on heating to  $1000^\circ\text{C}$  and  $\alpha' \rightarrow \beta$  on cooling; the second cycle gives  $\beta \rightarrow \alpha'$  (heating) and  $\alpha' \rightarrow \beta$  (cooling); during the third cycle a new modification  $\beta'$  appears on cooling. This work requires confirmation and extension, but the results already obtained would explain why the crystals of  $\beta_2\text{CaO} \cdot \text{SiO}_2$  grown for X-ray structure work have always shown inversion twinning as shown in Fig. 9 of our paper.

The successes obtained with the Verneuil technique reported by Zerfoss<sup>10</sup> are most encouraging. When using the "eutectic" method of crystallization, where the large number of components used in the melt often prevents any adequate forecast of the phase relations being made, it is particularly necessary to report chemical analyses of the resulting crystals. For instance, in the case quoted by Zerfoss, it seems very likely that a solid solution of  $\text{BaTiO}_3$  and  $\text{Ba}_2\text{Ti}_2\text{O}_7$  might be obtained, as is the case with the corresponding calcium compounds.

**Dr. D. R. Hale** (*Cleveland, Ohio*) (*communicated*): This paper reviews an ingenious process for growing quartz which may offer an improvement in growth rate and quality over that by which rock crystal was produced in nature. A modification of the Spezia method, however, using crystalline quartz as nutrient supply at an elevated temperature and a thermal gradient such that the region about the seed is at a lower temperature, has been used with considerable success at The Brush Development Co., Cleveland, Ohio.<sup>11</sup> More than half an ounce of quartz, free from cracks and veils, has been deposited on an untwinned *R*-plate having an area of about  $7\text{ cm}^2$  on a side. Contrary to the recommendation in the paper under discussion, the formation of spontaneous crusts on the wall of the chamber has been avoided as far as possible. The presence of such a coating, presenting a large area of rhombohedral quartz on which quartz can deposit from the supersaturated solution, may be helpful as a means to keep

<sup>8</sup> Sirota, *J. Tech. Physics, U.S.S.R.*, 1948, **18**, 1136.

<sup>9</sup> Trommel, *Naturwiss.* (to be published).

<sup>10</sup> This Discussion.

<sup>11</sup> Hale, *Science*, 1948, **107**, 393.

the supersaturation from reaching high values when the more soluble vitreous silica is used. Skeletal, drusy or other irregular types of deposition on the seed imply the effect of a highly supersaturated condition, assuming that the solution is clean and not strongly agitated. A well-controlled crystal-growing system should preferably avoid all spontaneous nucleation, which is the recognized ideal in growing the usual types of easily soluble substances.

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## V. CONCLUDING REMARKS

**Dr. C. W. Bunn** (*I.C.I., Plastics*) said: In looking at the Discussion as a whole, and at the relations between theoretical and experimental contributions, certain things seem to me to stand out. In the first place, I am agreeably surprised at the general agreement that a perfect crystal bounded by simple faces probably would not grow at all. I suggested this as an inference from experimental evidence, but hardly expected it to pass unchallenged; actually it appears that there are theoretical grounds for expecting this to be the case. So far, theory and experiment are in accord. My other outstanding impression about the theoretical work is that much of it is based on equilibrium considerations; yet crystal growth is, of course, not an equilibrium affair. I do not suggest that equilibrium considerations are irrelevant, but I think we should not assume that the conclusions from equilibrium considerations apply as they stand to actual crystal growth problems; or, at any rate, not to rapid growth. As they stand, they are likely to apply most closely to very slow growth. For the phenomena of rapid growth we need a dynamic theory, which treats crystal growth as a progressive event, takes into account the movements of the molecules in vapour, solution or melt, and considers how these movements influence the sites taken up on deposition and thus determine the character of the new surface on which further deposition is to take place.

If we accept the thesis that perfect crystals bounded by low-index faces do not grow at any reasonable supersaturation, we are faced by the problem of accounting for the fact that crystals do actually grow, even at very low supersaturations. There are two obvious solutions; one is that real crystals are not perfect, the other is that the surfaces on which deposition occurs are not low-index surfaces; and both these conceptions have figured in our discussions. No doubt both factors play a part in determining the rates at which crystals grow, and we have to enquire what is their relative importance in a variety of circumstances.

Turning to the experimental papers, they fall fairly sharply into two classes—those dealing with rapid growth and those dealing with extremely slow growth. The former are relevant to the industries which produce crystalline substances in large quantities and are necessarily concerned with rapid crystal growth; the study of the rate of nucleus formation and the rate of crystal growth is directed towards maximum production and control of grain size and shape. The latter are the concern of the industries which make large perfect crystals for optical prisms or piezoelectric elements, and must necessarily grow their crystals very slowly in carefully controlled conditions.

How are the industrial, experimental and theoretical aspects to be linked up? It seems to me that it is for problems of rapid growth from strongly supersaturated solution that a dynamic theory of crystal growth is most needed, and that since in rapid growth deposition apparently occurs on high-index surfaces at the edges of spreading layers, the central problem is the study of the factors which keep high-index surfaces alive. On the other hand, in very slow growth from slightly supersaturated solutions there is time for high-index surfaces to heal (that is, for the depositing molecules to go on to sites which give rise to low-index surfaces); in these circumstances, it is likely that imperfections play a dominant role in controlling growth; here, too, the theoretical approach based on equilibrium considerations is likely to be more directly applicable.

**Mr. P. R. Rowland** (*London*) said: The general view of the meeting seems to be that the gap between the theoretical and experimental approaches has been too wide. To the author, the reason for this seems obvious. As a colleague

remarked, "The subject is still in the alchemical stage." As an experimentalist the author feels that it is asking too much of the theoretical worker to provide even rough theories at this point of development. The information available is too meagre and the possible complications too many. However, we might have asked for guidance concerning the lines along which further work may be conducted. The author would therefore like to put forward a few opinions, though with some trepidation, since Prof. Stranski appears to have been following the course proposed and may already have forestalled them.

First, the subject should be subdivided under the following headings: (i) Growth from vapour. (ii) Growth from melt. (iii) Growth from solution. (iv) Growth by phase change in the solid state. Further subdivision according to whether the crystal is held together by ionic, homopolar, van der Waals' or metallic forces also seems desirable.

Two aims should be borne in mind:

(a) To provide a picture of the structures of the growing surfaces, meaning by "picture" the sort of information which is imparted by describing, say, methane or long conjugated chains in terms of  $\sigma$  bonds, etc.

(b) A similar picture of the medium from which the crystal is growing, with due regard to the fact that the situation is dynamic and not static.

The difficulty of supplying (b) will increase as we proceed from (i) above to (iii) ((iv) is a special case). In the case of growth from solution (a) and (b) may not be separable. For instance, when growing  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  crystals there may be quite a large growing region, in passing through which  $\text{Cu}(\text{H}_2\text{O})_4^{++}$ ,  $\text{SO}_4^{--}$  ions and water molecules gradually pass from a more or less random distribution to become units of a lattice correctly spaced and rotationally orientated. It would be difficult to say which point is the surface of the growing crystal. This picture is in fact supported by the observation that growth of good crystals from aqueous solution is only usually possible with hydrated substances, while growth from the melt seems to be the most successful way of producing really large perfect crystals. Can growth from solution be regarded as growth from a highly impure melt?

However, it would seem wiser to start with the simplest systems (a) above. Crystallization may then be regarded as a heterogeneous reaction and it is essential that we learn as much as possible about the surface of the substrate. The author regards the technique of forming spherical single crystals and studying reactions on their surfaces as a powerful tool in the experimental study of solid surfaces. Prof. Stranski has used it to study crystal growth itself.

It is suggested that progress from such beginnings may be made by carrying out work on the following lines:

1. Experiments to determine the behaviour of the surface of single crystal spheres towards various reagents, e.g., the vapour of the crystal substance, adsorbates, substances of varying electronegativity, polar substances, solvents, etc. The only way to ensure a really clean surface is to heat it in a vacuum and valuable information may be obtained by repeating some of J. K. Roberts's work with single crystal wires.

2. Experiments with spheres at temperatures very near their melting points to determine the mobility of surface layers. Growth from the vapour under these conditions, especially in cases where the gas phase could be made very dense, may give hints on the mechanisms of growth from the melt.

3. Growth of solvated crystals from the melt may throw light on growth from solution.

4. Dr. Bunn has shown how much is to be learnt by the direct observation of growing crystals. In the electron microscope and interferometry as developed by Prof. Tolansky and his school, we now have methods which enable us to observe almost down to molecular dimensions. Though they have limitations, obvious and otherwise, the author is sure that if the attempt were made to adapt them to the study of growing crystals, at least some confusion would be removed. The central problems could be recognized and attacked.

To sum up, the theoretical physicist will only have a fair chance of getting to grips with the problem when the experimentalist has revealed what its essentials are. It is suggested that the best line of approach is to begin with a systematic study of crystal surfaces by both direct and indirect methods.

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\* The references in heavy type indicate papers submitted for discussion







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A GENERAL DISCUSSION  
ON  
**LIPID-PROTEINS**

GENERAL INTRODUCTION

A GENERAL DISCUSSION on Lipid-proteins was held in the Department of Pharmacology, Birmingham University (by kind permission of Prof. A. C. Frazer) from the 29th to the 31st August, 1949. The Chair was taken by Prof. E. K. Rideal, F.R.S. About 117 members and guests were present; among the distinguished overseas guests Prof. E. K. Rideal welcomed the following :—

Mr. W. McD. Armstrong (Dublin), Dr. P. C. Blokker (Amsterdam), Dr. and Mrs. H. L. Booij (Leiden), Prof. E. Chargaff (New York), Prof. S. Claesson (Uppsala), Prof. A. Claude (Brussels), Prof. E. J. Cohn (Harvard), Dr. D. G. Dervichian (Paris), Dr. J. P. Dustin (Brussels), Dr. P. Dustin (Brussels), Dr. J. T. Edsall (Harvard), Dr. G. Ehrensverd (Stockholm), Dr. J. G. Faber (New York), Dr. M. Faber (New York), Prof. A. Frey-Wyssling (Zurich), Dr. W. Gaade (Amsterdam), Dr. J. M. Gillespie (Australia), Prof. F. Haurowitz (Bloomington, Ind.), Dr. and Mrs. E. Havinga (Leiden), Prof. M. Heidelberger (New York), Dr. O. Hoffmann-Ostenhof (Vienna), Dr. M. Joly (Paris), Dr. Jourovsky (Brussels), Dr. W. Kauzmann (U.S.A.), Prof. C. H. Li (Berkeley, Cal.), Prof. J. Murray Luck (Stanford, Cal.), Prof. M. Macheboeuf (Paris), Dr. Meduski (Lodz), Dr. D. H. Moore (U.S.N., London), Dr. and Mrs. E. Neuzil (Bordeaux), Prof. W. Niermiero (Lodz), Prof. J. M. O'Connor (Dublin), Miss M. C. Pangborn (New York), Prof. T. R. Parsons (Nigeria), Mlle. M. Raison (Paris), Prof. F. Tayeau (Bordeaux), Dr. E. C. Wassink (Wagenigen).



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# STRUCTURAL ASPECT OF LIPO-PROTEIN ASSOCIATION

By D. G. DERVICHIAN

*Received 2nd June, 1949*

From a detailed analysis of all the available work done on the association between proteins and different colloidal electrolytes, general and consistent conclusions could be derived concerning the simultaneous influence of pH, salt concentration and proportions of the two constituents; the important point being that these different factors intervene in a similar and complementary way. From this and other considerations, it is concluded that the interaction is purely ionic. The necessity of bringing in van der Waals' forces, in some cases, results from the binding together of the molecules in the colloidal electrolyte micelle.

The nature of the lipid-lipid associations is discussed from the point of view of mixed micelles. If their ionic behaviour is considered, natural lipo-proteins are similar to the artificial associations of proteins with ionic colloids. If the extraction of lipids is considered, natural lipo-proteins show the characteristics of the artificial lipid-lipid associations.

A tentative structure is proposed for the natural lipo-proteins in solution. Lipids would form separate mixed micelles in which the non-ionic are solubilized by the ionic. It is further postulated that a purely ionic interaction takes place between these mixed lipidic micelles and the protein particles as well as with all other small ions present.

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The natural lipo-proteins, i.e. those encountered in living organisms, contain ionic lipids (phospholipids, fatty acids) and non-ionic lipids (esters of cholesterol, cholesterol, glycerides). First will be examined the protein-ionic lipid association. No doubt it is the protein-lecithin interaction which is the most instructive from the point of view of the natural lipo-proteins. But, if one examines and compares simply the experimental facts, very satisfactory correlations are found in the behaviour of lecithin alone and of the different associations: protein-lecithin, protein-fatty acids, protein-synthetic detergents and, in a general way, protein-colloidal electrolytes such as gum arabic, nucleic acid, dyes. It appears that non-ionic lipids cannot associate by themselves with proteins, while they can associate with the other ionic lipids. The study of those lipid-lipid associations coming next will introduce some structural considerations which can explain some of the specific behaviour of the natural lipo-proteins. Finally, the behaviour and properties of natural lipo-proteins will be examined in the light of the conclusions of the preceding studies.

## Protein-Ionic Lipid Interaction

The precipitation of protein-lipid systems gives certain information on the interaction of these two classes of substances. In certain cases, instead of ordinary precipitation or flocculation, a fluid phase separates which is called coacervate.<sup>1</sup>

<sup>1</sup> Dervichian, *Research*, 1949, 2, 210.



**(a) Mutual Influence of pH and Proportion on Precipitation.**

—Concerning zones of precipitation at certain proportions of the constituents and the influence of pH on these proportions, the same peculiarities are found either in the protein-lecithin interaction<sup>2-6</sup> or in the interaction of proteins with other ionic lipids,<sup>5, 7</sup> detergents<sup>8-14</sup> colloidal electrolytes such as gum arabic,<sup>15, 16</sup> or nucleic acid,<sup>16, 17</sup> and dyes.<sup>15-20</sup> The behaviour of anionic detergents on the one hand and cationic detergents on the other are symmetric about the isoelectric point of the protein on which they act.

**(b) Influence of Electrolytes.**—Even with lecithin alone, the precipitation by an acid is inhibited by salts.<sup>3</sup> The presence of neutral salts also inhibits the precipitation of lecithin by proteins.<sup>3</sup> More exactly, to precipitate lecithin by protein, the acidity of the medium must be higher the more neutral salt is present.<sup>2</sup> This property is also found in interactions with other colloidal electrolytes.<sup>7</sup> With anionic detergents the amount of detergent necessary to produce complete precipitation increases with the amount of added electrolyte.<sup>10</sup> The same phenomenon is observed with cationic detergents in basic medium.<sup>11, 14</sup> Above the isoelectric point, precipitation by an anionic detergent is only possible in the presence of a considerable quantity of salt. In addition, the ratio detergent/protein in the precipitate is nearly ten times its value in acid medium in the absence of salt.<sup>21</sup> Finally, coacervation of gelatin by gum arabic is strongly influenced by the presence of salts.<sup>15</sup> For example, 0.35 % KCl is sufficient to suppress the separation.

One sees that pH and saline concentration play an identical role and this in accord with the fact that, with proteins alone, the addition of salts causes an increase in the pH of the solution if the initial pH is less than that of the isoelectric point, and a decrease in pH if the initial pH is greater than that of the isoelectric point.<sup>22</sup>

It should be pointed out also that the protein and the other colloidal electrolyte each carries its gegenions, which explains the fact that an excess of one or the other influences the precipitation in the same manner as ions of added salt or OH<sup>-</sup> or H<sup>+</sup> ion (pH).

<sup>2</sup> Mayer and Terroine, *Compt. rend. Soc. Biol.*, 1907, **62**, 398.

<sup>3</sup> Handovsky and Wagner, *Biochem. Z.*, 1911, **31**, 32.

<sup>4</sup> Parsons, *Biochem. J.*, 1928, **22**, 800.

<sup>5</sup> Przylecki and Hober, *Biochem. Z.*, 1936, **288**, 303.

<sup>6</sup> Feinschmidt, *Biochem. Z.*, 1912, **38**, 244.

<sup>7</sup> Matsumura, *Kolloid-Z.*, 1923, **32**, 171.

<sup>8</sup> Bull and Neurath, *J. Biol. Chem.*, 1937, **118**, 163.

<sup>9</sup> Putnam and Neurath, *J. Amer. Chem. Soc.*, 1944, **66**, 692.

<sup>10</sup> Pankhurst and Smith, *Trans. Faraday Soc.*, 1945, **41**, 630.

<sup>11</sup> Schmidt, *Z. Physiol. Chem.*, 1943, **277**, 117.

<sup>12</sup> Elkes and Finean, in *Surface Chemistry* (Butterworth, London, 1949), p. 281.

<sup>13</sup> Dervichian and Magnant, *Bull. Soc. Chim. biol.*, 1947, **29**, 655.

<sup>14</sup> Polonovski and Macheboeuf, *Ann. Inst. Pasteur*, 1948, **74**, 196.

<sup>15</sup> Bungenberg de Jong and Dekker, *Kolloid. Beih.*, 1935, **43**, 143; 1936, **43**,

213.

<sup>16</sup> Dervichian and Magnant, *Bull. Soc. Chim. biol.*, 1947, **29**, 660-666.

<sup>17</sup> Bungenberg de Jong and Hoskam, *Proc. Ned. Akad. Wetensch.*, 1942,

**45**, 387.

<sup>18</sup> Mathews, *Amer. J. Physiol.*, 1898, **1**, 445.

<sup>19</sup> Chapman, Greenberg and Schmidt, *J. Biol. Chem.*, 1927, **72**, 707.

<sup>20</sup> Rawling and Schmidt, *ibid.*, 1929, **82**, 709.

<sup>21</sup> Pankhurst and Smith, *Trans. Faraday Soc.*, 1944, **40**, 565.

<sup>22</sup> Schmidt, *The Chemistry of Amino Acids and Proteins* (Charles C. Thomas, 1945), p. 1209.

(c) **Interaction in Solution.**—The separation of proteins by other colloidal electrolytes can occur only in a pH region such that the net charge of the protein is opposite to that of the precipitating agent. There are, nevertheless, interactions between the two constituents in regions where the net charges are of the same sign and where the constituents remain in solution. These interactions have been demonstrated mostly by electrophoresis studies.<sup>23, 24</sup> Well-defined boundaries appear which correspond to the migration of the associated protein and detergent. These interactions have also been demonstrated by measurements of rotation of plane polarized light, surface tension and pH.<sup>25</sup> More recently, Pankhurst<sup>26</sup> has added evidence based on viscosity measurements.

Interactions between acid or basic dyes and proteins outside the pH zone of precipitation has been demonstrated by measurement of the shift of absorption bands and determination of the quantity of dye held by the protein in dialysis equilibrium.<sup>27</sup>

(d) **Nature of Protein-Ionic Lipid Interaction.**—Mayer and Terroine<sup>2</sup> established that in an electric field the charge (positive) of the lecithin-albumin complex was opposite to that of lecithin alone. It was Putnam and Neurath<sup>9</sup> who were the first to realize the connection between the maximum quantity of detergent bound and the total acid-binding capacity of the protein. They concluded that precipitation was produced by electrostatic forces between the ionized groups of the two constituents. In studying the electrophoresis of small drops of gelatin-gum arabic coacervate, Bungenberg de Jong and Dekker<sup>15</sup> found they were drawn to the positive or negative pole according to the relative amount of gum arabic. For each pH the change of sign occurs in the neighbourhood of the maximum separation. It appears then as if coacervation were connected to the mutual neutralization of the charges of the two colloids.

In what form is the lipid bound to the protein in the precipitate? The method of titration used by Parson<sup>4</sup> might give us some information on this point. The lecithin present in the precipitate was titrated by dissolving in it a lipo-soluble dye (but not water-soluble). This dye remained in the associated lipid. There is strong evidence that lipo-soluble dyes (like other lipo-soluble substances) can only be dissolved between the paraffinic chains of lecithin or detergent when these molecules are associated in micelles.<sup>28-30</sup> The work of Parsons tends to prove that in the protein-lecithin precipitate the lecithin is associated in the form of lamellar micelles. The fact that this micellar structure is necessary is shown by the observation that proteins cannot be precipitated by detergents (anionic or cationic) having less than 10 carbon atoms. Besides, it is well known that, at ordinary temperature, micelle structure exists only in solutions of ionic lipids having more than 8 carbon atoms in their chain.

To satisfy all these conditions, i.e. ionic interaction between lipids and proteins, necessity of micellar state for the lipid and the appearance

<sup>23</sup> Lundgren, Elam and O'Connell, *J. Biol. Chem.*, 1943, **149**, 183.

<sup>24</sup> Putnam and Neurath, *ibid.*, 1945, **159**, 195.

<sup>25</sup> Desreux and Fabry, *Bull. Soc. Chem. biol.*, 1946, **28**, 478.

<sup>26</sup> Pankhurst, *ibid.*, 1949, **31**.

<sup>27</sup> Klotz, *Chem. Rev.*, 1947, **41**, 373.

<sup>28</sup> Dervichian and Magnant, *Compt. rend. Soc. Biol.*, 1946, **140**, 95.

<sup>29</sup> Kiessig and Philippoff, *Naturwiss.*, 1939, **27**, 593.

<sup>30</sup> Hughes, Sawger and Vinograd, *J. Chem. Physic.*, 1945, **13**, 131.

of paraffinic character in organic solvents,<sup>2, 31</sup> one could suggest that *in the precipitate* the double layer micelles of the lipid stick by their ionic faces to the ionic faces of the protein particles and thus constitute alternate layers of lipid and protein.<sup>32, 33</sup>

The structure proposed here might not seem to differ from that of Pankhurst and Smith.<sup>31</sup> In fact it originates from a different point of view. The interaction of the lipid micelle *as a whole* has to be considered at the moment of precipitation, starting from the principle that no lipid molecules can adsorb in a single layer without the double layer lattice being built up and precipitation following. One could as well say that the micelle of the detergent fixes the protein particles by its two external ionic faces (and vice versa) and that the ionic lattice (Hartley) being thus formed, similarly to the crystal of fatty substances, the whole precipitates.

Should the "dissolved complexes" be considered as formed of complex particles with lipid molecules sticking to the protein molecules as they are in the precipitate? To see the problem clearly one must not lose sight of a very trivial fact: ionic combination in solution does not mean aggregation of oppositely charged ions in one particle. Proportions are stoichiometric whether the reaction gives rise to aggregation and precipitation or to a dissociated compound which remains in solution.

It is generally admitted that the length of the lipid or detergent chain serves to increase the forces of interaction between the lipid and the protein in a discontinuous way when there are more than eight carbons in the lipid chain.<sup>24, 34, 35</sup> Some see here a specific action. Thus two factors would determine the force of the interaction: an electrostatic attraction due to oppositely charged ions, and van der Waals' forces. This is also the conclusion arrived at by Klotz<sup>27</sup> concerning the bonds between proteins and dyes. The basis of this conclusion is the difference in behaviour between dyes having one charged group and those having two or three charged groups. However, the point should be considered that with amphipatic molecules the tendency to form micelles diminishes with the increasing number of solubilizing groups. It seems reasonable to think that the necessity of bringing in van der Waals' forces is not concerned with the bond between the protein and the lipid, but with the linkages which hold together the lipid molecules *in the lipid micelle* in the neighbourhood of the protein particle. The interaction between the lipid micelle as a whole and the protein particle is purely ionic.

The "soluble compounds" are formed in the pH region where the protein and the lipid have the same charge. How can ionic interactions take place under such conditions? The answer is given by Schwert, Putnam and Briggs<sup>36</sup> who admit that such interactions may take place in a pH range where the two components have a net charge of the same sign, provided there exist ionized groups on the protein opposite in sign to that of the net charge. Klotz<sup>27</sup> reached the same conclusion concerning the interaction between dyes and proteins.

<sup>31</sup> Pankhurst, in *Surface Chemistry* (Butterworth, London, 1949), p. 109.

<sup>32</sup> Elkes, Frazer, Schulman and Stewart, *Proc. Roy. Soc. A*, 1945, **184**, 102.

<sup>33</sup> Palmer, Schmitt and Chargaff, *J. Cell. Comp. Physiol.*, 1941, **18**, 43.

<sup>34</sup> McMeekin, *Federation Proc.*, 1942, **1**, 125.

<sup>35</sup> Boyer, Ballou and Luck, *J. Biol. Chem.*, 1947, **167**, 407.

<sup>36</sup> Schwert, Putnam and Briggs, *Arch. Biochem.*, 1944, **4**, 371.

But how is it that the bond is purely ionic, when electrophoresis shows sharp boundaries indicating migration of the two associated constituents with no dissociation? Such migration toward the same electrode of an anion and a cation have been found by Hartley, Collie and Samis<sup>37</sup> with a small ion like  $\text{Br}^-$  associated with a cationic detergent in cetylpyridinium bromide. The bromine ion mobility is so much reduced that it becomes actually negative. More bromine is carried in the reverse direction attached to the micelles than travels unattached in the normal direction. The adherence of gegenions to the micelle is due to the attraction of the free gegenions into a dense atmosphere around the micelle.<sup>38</sup> Thus emerge the particular conditions which exist at the surface of the micelles of the colloidal electrolytes, due to the high concentration of packed charges. However, in protein-detergent association, conditions are even more favourable than those of cetylpyridinium bromide because the electrophoresis is carried out at a pH where both substances have net charges of the same sign and both have micelle structure.

One might think that the interaction is localized at the positive ions of the protein (e.g. protein-anionic lipid interaction at an alkaline pH), only single molecules of the lipid being able to approach and not compact micelles because of the repulsion of the negative protein ions. This is where van der Waals' forces, acting between paraffinic chains in the lipid micelle, must come in. This means that it requires more energy to keep paraffin chains separated in the presence of water than to draw some of the anionic groups of the detergent near to the anionic groups of the protein. Besides, if groups of the same sign always repelled one another, soaps would never crystallize nor show paracrystalline phases in water. In the lamellar structure of soap, the  $\text{COO}^-$  groups are face to face in what Hartley calls an ionic lattice. No doubt this ionic lattice is stabilized by the presence of small  $\text{Na}^+$  ions. In the present case, the small cations of salt present in the system or even the amino groups of the protein themselves would play the same role.

Whatever is the mechanism, we can at least say that it is sufficient to have a few points of attraction between the positive groups of the protein and the negative groups of the lipid for the whole lipid micelle to be held in the neighbourhood of the protein molecule. If the solution is acid with respect to the isoelectric point of the protein, the ionic interactions become overwhelmingly important and there is precipitation. The same reasoning can be applied *mutatis mutandis* to cationic lipid-protein interaction.

In coacervation of gelatin or haemoglobin by gum arabic, excess of one constituent causes both constituents to pass into solution. It cannot be seen how the acid groups of gum arabic could be fixed individually to a greater or smaller number of the amino groups of the protein, nor could be understood how a second layer of gum arabic could be formed, making the surface soluble. Such assumptions have been made to explain the same phenomenon with detergents.<sup>21, 39</sup>

The influence of the quantity of electrolyte present either on the pH of optimum precipitation or on the proportion of detergent

<sup>37</sup> Hartley, Collie and Samis, *Trans. Faraday Soc.*, 1936, **32**, 795.

<sup>38</sup> Hartley, *Aqueous Solutions of Paraffin-chain Salts* (Hermann et Cie, Paris, 1936).

<sup>39</sup> Lundgren, *Textile Res. J.*, 1945, **15**, 335.

precipitated shows that the whole phenomenon is a matter of neutralization of charge in a given volume element. This explanation is similar to that given in the case of ordinary electrolytes. This spatial neutralization, in which small and large ions take part, causes either precipitation or changes in velocity of migration in an electric field. We saw that the presence of salt inhibits precipitation. This effect of small ions may be duplicated by the lipid ions. An excess of detergent (carrying its gegenions) produces the same inhibitory effect by perturbing the ionic atmosphere. This appears as if the acid binding capacity of the protein were modified (see (a) and (b) above).

In the experiments of Pankhurst and Smith<sup>21</sup> at pH above the isoelectric pH, the quantity of detergent fixed is ten times the acid binding capacity. These authors (as has Steinhardt<sup>40</sup>) suggest that this quantity corresponds to fixation by the amide groups following shifting of their dissociation constants. This point of view can be reconciled with that presented here if, instead of fixation, we consider the effect of compensation of charge in each small volume element.

Other models have been proposed in which the bond is made by the paraffinic end of the detergent linking itself to the paraffinic groups of the protein. Such models have been proposed by Macheboeuf and Sandor,<sup>41</sup> by Dervichian,<sup>42</sup> and by Palmer.<sup>43</sup> The accumulated proofs, direct and indirect, of the ionic nature of the phenomena seems now to exclude such a possibility.

In the model here proposed there would not be fixation of the detergent to the protein. Fixation results in precipitation just as with ordinary ionic compounds. In solution, what we call a compound or a complex is dissociated, but the charge can be modified by the presence of  $H^+$  or  $OH^-$  ions (action of pH) or by other small ions of salts present. A reason for this effect to be so apparent is that the ions of the detergent and the protein are strongly concentrated at the surface of their respective micelles. The proximity of the charges introduced by the protein, by the fact that they are opposite to that of the lipid, could tend to ionize the detergent groups. Thus some ionic lipids, insoluble in water, are made soluble as soaps are in the presence of alkaline metal ions.

### Lipid-Lipid Associations

A whole series of associations in the bulk can be obtained in definite proportions of lipids in contact with water.<sup>44</sup> Generally speaking, it is the structure of the constituents which comes into play and not a particular substance (e.g. a molecule of a long-chain aliphatic compound associated with a steroid molecule). Consequently, in such an association, a molecule may replace another of a similar type.

A systematic study of a great number of pairs of lipids, the one soluble and the other insoluble, have allowed detection of a gradation going from simple swelling to complete dispersion.<sup>45</sup> In complete

<sup>40</sup> Steinhardt, *Ann. Rev. Biochem.*, 1945, 14, 145.

<sup>41</sup> Macheboeuf and Sandor, *Bull. Soc. Chim. biol.*, 1932, 14, 1168.

<sup>42</sup> Dervichian, *J. Chim. physique*, 1941, 38, 59; *J. Chem. Physics*, 1943, 11, 219.

<sup>43</sup> Palmer, *J. Physic. Chem.*, 1944, 48, 12.

<sup>44</sup> Dervichian and Magnant, *Bull. Soc. Chim. biol.*, 1946, 28, 419; *Compt. rend. Soc. Biol.*, 1946, 140, 94.

<sup>45</sup> Dervichian, *Trans. Faraday Soc.*, 1946, 42B, 180.

dispersion the two constituents are associated in mixed micelles. Substances insoluble like cholesteryl oleate can thus be dispersed in water.<sup>46</sup>

The manner of association of molecules of lipids is known. Myelinic figures formed by such associations have a clearly stratified structure<sup>47, 48</sup> composed of double lipid layers separated by layers of several molecules of water. In each lipid layer, the different types of molecule are oriented side by side, their polar groups directed toward the water and their paraffinic chains facing the paraffin chains of the adjoining layer, likewise oriented toward the water. The same double layer structure is preserved in other forms of swelling and one is led to suppose that it is preserved also in the separate micelles when completely dispersed.

The association cannot be explained on purely chemical grounds, but from a crystallographic point of view. The principal point is that association cannot be realized except in the presence of water and that water itself must be regarded as one of the structural constituents. Thus is explained the failure of the authors who have tried to isolate complexes in the absence of water.<sup>49</sup>

### Natural Lipo-Proteins

A general examination of lipo-proteins shows a striking parallelism to that of protein-ionic lipid and lipid-lipid associations. We are speaking here only of lipids masked in serum or the cells and not those which are found in the form of microscopic or ultramicroscopic particles (for details, see for example<sup>50</sup>).

**(a) Ionic Behaviour.**—The lipo-proteins of serum precipitate at a pH lower than the isoelectric pH and go into solution again when the pH is raised to 7,<sup>51</sup> exactly like anionic lipid-protein associations.

The fact that natural lipo-proteins are not dissociated in the electrical field<sup>52, 53</sup> does not, as thought by Cohen and Chargaff,<sup>53</sup> plead against the assumption of a simple saline bond between proteins and phospholipids. It was shown, that not only in protein-detergent mixtures but even in cetylpyridinium bromide both constituents or ions migrate together.

**(b) Extraction of Lipids.**—From the point of view of extraction, natural lipo-proteins show predominantly the characteristics of lipid-lipid associations. It is not a matter of solubility accident, contrary to the idea of Chargaff,<sup>54</sup> "that substances as disparate as cephalin and cholesterol" are extracted jointly. This type of pair gives the best lipid-lipid association.

Extraction by ether can only be accomplished after dehydration by cold alcohol for example (Hardy and Gardiner method). This is

<sup>46</sup> Valette and Cavier, *Bull. Soc. Chim. biol.*, 1938, **20**, 1256.

<sup>47</sup> Nageotte, *Compt. rend.*, 1927, **185**, 1021.

<sup>48</sup> Browaes and Dervichian, *Compt. rend. Soc. Biol.*, 1946, **140**, 136.

<sup>49</sup> Partington, *J. Chem. Soc.*, 1911, **99**, 313, 318.

<sup>50</sup> Elkes, Frazer and Stewart, *J. Physiol.*, 1939, **95**, 68. Frazer, *Trans. Faraday Soc.*, 1941, **37**, 125.

<sup>51</sup> Macheboeuf, *Bull. Soc. Chim. biol.*, 1929, **11**, 268 and 485.

<sup>52</sup> Macheboeuf, Delsal, Lepine and Giuntini, *Ann. Inst. Pasteur*, 1943, **69**, 321.

<sup>53</sup> Cohen and Chargaff, *Biochem. J.*, 1940, **136**, 243.

<sup>54</sup> Chargaff, *Advances in Protein Chemistry* (1944), **1**, 18.

identical with the behaviour of lipids alone or lipid-lipid associations which cannot be extracted by ether as long as they remain dispersed in water in the form of micelles. The lowering of the temperature reconstitutes the ionic lattice<sup>45</sup> (Krafft point) even in the presence of water and makes association impossible (e.g. myelinic forms do not develop). Extraction by ether becomes then possible. This explains the effectiveness of the method of McFarlane.<sup>55</sup> The presence of alcohol destroys the micelle structure<sup>56</sup> and prevents lipid association. This explains why the extraction of purely lipidic associations by ether is not possible at ordinary temperature except in the presence of alcohol,<sup>57, 58</sup> and allows us to understand why the addition of alcohol to serum helps the extraction of lipids by ether.<sup>41</sup>

Macheboeuf and Tayeau<sup>59</sup> showed that the addition of soap to serum permits extraction by ether. In addition it can be seen in the curves of these authors that the maximum of extraction is realized for a soap/protein ratio of 0.4 which is exactly that found as binding capacity of proteins towards detergents.<sup>9</sup> There is therefore substitution from the point of view of ionic interactions: we have seen that fatty acids themselves associate with proteins. The quantity of extracted lipids then diminishes with increasing proportion of soap. This might be due to the association of the excess of soap with the separated lipids keeping them in solution. The action of heparin on the extraction<sup>60</sup> is similar to that of soap.

**(c) Lipid-Lipid Association.**—The fact that cholesterol and phospholipids are liberated simultaneously by the action of soap seems to indicate that there is substitution of soap micelles for the mixed cholesterol-phospholipid micelles as a whole. In addition, the specific extraction of cholesterol alone by substitution of molecules of similar structure (saponin with a steroid structure<sup>61</sup> or sodium dehydroabietate<sup>62</sup>) reminds us of the typical specific conditions of the purely lipidic associations and seems to show that cholesterol is directly bound with the phospholipids in mixed micelles.

Tayeau<sup>61</sup> had already been led to the conclusion that cholesterol is mainly bound to the phosphatides and that phosphatides might form the link between cholesterol and the proteins. White<sup>63</sup> in 1908 had already foreseen that cholesterol is normally present in the tissues of animal in a state of "weak combination" with fatty acids and lecithin, forming liquid crystals.

The suggestion has been made that at least a small part of the lipids were bound to the protein by stronger bonds. This conclusion is based on the fact that part of the lipids cannot be extracted by the above-mentioned methods. In fact, lipids might be included in the structure of the protein. Nevertheless, it should be pointed out that this behaviour is not particular to natural lipo-proteins, as it is also found with ionic lipids alone or artificially associated to proteins.<sup>5, 64</sup>

<sup>55</sup> McFarlane, *Nature*, 1942, **149**, 439.

<sup>56</sup> Ward, *Proc. Roy. Soc. A*, 1940, **176**, 412.

<sup>57</sup> Delezenne and Fourneau, *Bull. Soc. chim.*, 1914, **15**, 421.

<sup>58</sup> Baranger, *Ann. Physiol.* 1937, **13**, 341.

<sup>59</sup> Macheboeuf and Tayeau, *Bull. Soc. Chim. biol.*, 1941, **23**, 49.

<sup>60</sup> Chargaff, Ziff and Cohen, *J. Biol. Chem.*, 1940, **136**, 257.

<sup>61</sup> Tayeau, *Bull. Soc. Chim. biol.*, 1944, **26**, 287.

<sup>62</sup> Macheboeuf and Rebeyrotte, *ibid.*, **26**, 475.

<sup>63</sup> White, *Medic. Chron.*, 1908, p. 47.

<sup>64</sup> Long, *J. Amer. Chem. Soc.*, 1908, **30**, 881.

## Conclusion and Tentative Structure of Natural Lipo-Proteins

It is easy to conceive that with as many varied constituents as we find in the cell or in serum, associations may come undone and be rebuilt in new compositions in the course of extraction. Water being the indispensable structural constituent, if one tries to "extract" by first dehydrating the system, the association may vanish. In other cases the extracted complex may well be an artefact. This means that the lipids and the proteins may well be associated in the aqueous phase of the cell or the plasma, differently and with some other constituents than those which come out by precipitation.

The solubilization of esters of cholesterol and glycerides, their simultaneous occurrence with phospholipids and their mode of extraction, in particular their extraction by substitution of a molecule of similar form, all suggest that non-ionic lipids could be associated with ionic lipids in mixed micelles which can be dispersed in aqueous solution. The identical behaviour of natural lipo-proteins and artificial lipid-protein associations favours an interaction of ionic nature between protein particles and lipid micelles. This interaction can take place, as in all ionic interactions *in solution*, between dissociated components which remain separated. The union of these particles gives rise to precipitation. This is not in contradiction to the evidence of associated migration in an electric field or simultaneous sedimentation<sup>53</sup> in a gravitational field.

Association in mixed micelles increase the solubility of lipids. In addition, ionization of the protein can, because of the interaction, increase the ionization of the lipid and therefore increase its solubility and dispersion.

The following structure is compatible with all these properties. In solution, proteins and associated lipids constitute two sets of separated micelles. Interaction takes place between these two species of micelles and all other small ions present, producing neutralization of their charges in every small volume element of the solution.

This state would be represented in the isotropic media of the cells or the plasma. The alternating lipid and protein layer structure of the myelinic nerve sheath,<sup>65, 66</sup> formed by the same type of elements but arranged regularly, would represent the other extreme case. Thus the lipo-protein solution would be to the paracrystalline nerve sheath what the isotropic soap solution is to the middle-soap or neat-soap anisotropic phases.<sup>45</sup>

I wish to thank Mr. R. S. Titchen for his help in writing this paper in English.

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Institut Pasteur,  
Paris.*

<sup>65</sup> Palmer and Schmitt, *Cold Spring Harbor, Symp. Quant. Biol.*, 1940, 8, 97.

<sup>66</sup> Elkes and Finean, in *Surface Chemistry* (Butterworths, London, 1949), p. 289.



## GENERAL DISCUSSION \*

**Dr. K. G. A. Pankhurst** (*London*) said: According to Dr. Dervichian's model the matrix of the complex is the detergent micelle, but we have found that it is possible to observe these phenomena at sub-micellar concentrations, e.g., with very dilute gelatin solutions at pH 2 in the absence of inorganic salt and *ca.*  $10^{-4}$  M dodecyl sodium sulphate, or even with octyl sodium sulphate. Any hypothesis to explain the formation of detergent-protein complexes must account for the observation that, as the detergent-protein ratio ( $D/P$ ) is increased the complexes become increasingly *lipophilic* (and may in certain circumstances be thrown out of solution as a precipitate or a coacervate) until a critical  $D/P$  is reached where water solubility is minimal, and that increasing  $D/P$  still further causes the complex to become increasingly *hydrophilic*. The model that I have suggested<sup>1</sup> has the protein as its matrix and does not require micelle formation for its initiation.

**Dr. D. G. Dervichian** † (*Paris*) said: Dr. Pankhurst thinks that interactions between proteins and lipids giving phase separation can occur below the critical concentration of micelle formation of the ionic lipid. I agree that interactions (i.e. charge neutralization in each element of volume) must take place between protein and lipid even if the latter is molecularly dispersed. But the point in question is that the best precipitants are the molecules containing more than 8 carbon atoms,<sup>24, 25</sup> higher concentrations of  $C_8$  or  $C_{10}$  being required as compared to the effective concentration of dodecyl sulphate. Dr. Pankhurst has shown us that very small concentrations of octyl sulphate do give coacervation with gelatin at pH 2. This is in accord with the general conclusions on the mutual influence of pH and proportion on precipitation.

It has also been shown<sup>24, 25, 26</sup> that the force of interaction between the lipid and the protein is considerably and suddenly increased when there are more than eight carbon atoms in the lipid chain. If the paraffinic chain of the detergent contributed *directly* to strengthen the bond with the protein (by van der Waals' forces), it would be difficult to understand the sudden increase of this binding energy with the appearance of the micellar dispersion above a certain chain length.

The view advanced in my paper is that the necessity of bringing in van der Waals' forces is due to the linkages which hold together the lipid molecules in the *lipid micelle*. This explains also the fact that with dyes having more than one charged group, the binding diminishes.<sup>27</sup> In fact, it is well known that the tendency to form micelles diminishes with molecules having more than one charged group.

Finally, the micellar structure of the lipidic constituents in natural lipo-proteins would explain particularly the solubilization of the insoluble lipids. This might be accomplished by the formation of purely lipidic mixed micelles.

**Dr. J. Elkes** (*Birmingham*) said: In keeping with Dr. Pankhurst's observations we, also, have observed the formation of protein-detergent complexes at submicellar concentrations of detergent. This was particularly apparent with a chromoprotein such as oxyhaemoglobin. Using  $10^{-4}$  M solution of sodium hexadecyl sulphate and dilute solutions of oxyhaemoglobin, the typical zoning phenomenon, showing both insoluble and soluble complexes, was regularly seen. A tentative structure for these complexes has been suggested.<sup>2</sup>

**Dr. D. G. Dervichian** (*Paris*) said: In the work to which Dr. Elkes refers the action of hexadecyl sulphate on oxyhaemoglobin has been observed at room temperature. The insoluble complexes were obtained at pH values below 6.1 and ranging down to pH 5.1. It is highly prob-

\* On preceding paper.

<sup>1</sup> This Discussion.

† Reference numbers here and in my later remarks are to my paper.

<sup>2</sup> Elkes and Finean, *Surface Chemistry* (Butterworth, 1949), p. 281.

able that at temperatures below  $30^{\circ}\text{C}$  and at acid pH values,  $10^{-4}\text{ M}$  is *not* a "submicellar concentration" (i.e. below the "critical concentration of micelle formation") for sodium hexadecyl sulphate.

This draws attention to a rather frequent error. That is to think that, for a given detergent, the critical concentration is independent of temperature and pH. First of all, for each detergent, there is a fairly sharply defined temperature above which the detergent is dispersed in the micellar form giving a colloidal solution and below which the system appears as a suspension of microcrystals in water. Above this temperature, the critical concentration of sodium hexadecyl sulphate (i.e. the maximum quantity of detergent soluble under the molecular dispersed form, saturation producing the appearance of micelles) is certainly higher than  $10^{-3}$ . Below this temperature, there is no more question of micelles, the dissolved molecules are in equilibrium with precipitated microcrystals and the saturation concentration (and not the "critical concentration") is certainly less than  $10^{-4}$ . The evidence that in the work quoted by Dr. Elkes the detergent at room temperature was below this particular temperature is given by the indication that the detergent "solution" was previously heated at  $40^{\circ}\text{C}$  before being pipetted into each tube.

Similarly, at a given temperature, decrease of pH below neutrality transforms the micellar solution again to a suspension of microcrystals. For example, the molecular solubility of potassium laurate was studied in my laboratory at  $23^{\circ}\text{C}$ , which is above the critical temperature. If for alkaline solutions the critical concentration is nearly  $10^{-3}$ , at pH 5 the solubility falls to rather less than  $10^{-4}\text{ M}$ . The experiments quoted by Dr. Elkes cannot therefore be invoked in support of Dr. Pankhurst's remark, particularly as far as the insoluble complexes (precipitate) are concerned.

As I have admitted in my reply to Dr. Pankhurst, interactions must take place in solution between protein and lipid even if the latter is molecularly dispersed. In my opinion, however, this interaction corresponds simply to charge neutralization in each element of volume of the solution. In support of this idea, Elkes and Finean's work was one of those I have quoted in my paper concerning the striking relation between pH and detergent/protein proportion.

**Dr. J. Elkes** (*Birmingham*) (*communicated*): Far from being unaware of the effects of either temperature or pH on the critical micellar concentration of detergent, detergent-buffer controls (without protein) were thought necessary and put up in each and every experiment.<sup>1</sup> Moreover in the paper referred to by Dr. Dervichian,<sup>1</sup> we give the concentration of detergent used as 0.13 %. It is at this high concentration that heating to  $40^{\circ}\text{C}$  was necessary to ensure "solvation". Lower detergent concentrations ( $10^{-4}\text{ M}$ ) were only employed in later unpublished experiments, and it is to these that we wish to refer.

**Dr. A. S. McFarlane** (*London*) said: Dr. Dervichian has found a general similarity in natural and artificial lipo-proteins, including those of serum. I have failed entirely to prepare artificial serum lipo-proteins using a variety of lipids—cholesterol, cholesteryl oleate, triolein, phospholipids, and either defatted serum, or a serum of low natural lipid content. Either the emulsion is not optically clear, or if it is, as for example, when using lecithin, the lipid does not migrate with any of the protein components seen in the electrophoresis apparatus. I would like to know if Dr. Dervichian or anyone here has been successful in doing this.

**Dr. D. G. Dervichian** (*Paris*) said: Non-ionic lipids cannot be dispersed in the presence of serum. It is possible, however, to get complete dispersion by associating substances of different molecular structure in proportions which vary with the nature of each constituent. Yet these dispersions cannot be obtained by simple addition of the different constituents to water or serum: very intimate mixing approaching the molecular scale must be realized.<sup>44, 45, 46</sup>

Regarding migration in an electric field, it is certainly influenced by pH and the proportions of lipid and protein. Boundaries common to the protein and the detergent have been observed by some authors.<sup>23, 24</sup>

**Dr. J. A. V. Butler** (*London*) said: I should like to make an elementary point. Both the speakers have referred to "protein" without specifying what protein they mean. Everyone will agree that proteins vary enormously in their properties and are highly specific in their behaviour. Surely it is desirable always to state *what* protein is referred to. We can hardly expect all proteins to act similarly, even with respect to detergents. The fact that one protein behaves in one way and another in another way is not very surprising.

**Prof. D. G. Dervichian** (*Paris*) said: The whole interest of the comparison of the results obtained by different authors<sup>2-27</sup> resides precisely in the point that the same behaviour has been observed whatever the specified protein or the ionic lipid. General and consistent conclusions can definitely be deduced concerning the related influence of pH, concentration of electrolytes, and proportions of protein and ionic lipid.

**Dr. J. T. Edsall** (*Harvard*) said: Dr. Dervichian has emphasized the ionic character of the surface of protein molecules. However, in addition to the positively and negatively charged ionic groups, there are also numerous non-polar side-chains, so that an ion such as an alkyl sulphate would presumably adhere to a protein surface with the sulphate radical in proximity to a positively charged group on the protein, while the alkyl side-chain could lie next to some adjoining non-polar side-chains of the protein. This seems a much more likely configuration than one in which the alkyl group projects out into the solvent, away from the protein surface.

I should like to second what Dr. Butler has said about the specificity of the reactions of different proteins with lipids and related substances. I do not believe that the profound difference between serum albumin and  $\gamma$ -globulin, for example, in their interactions with dyes and with fatty acid anions, can be explained by any simple electrostatic mechanism.  $\gamma$ -Globulin contains numerous cationic groups, which should be capable of binding added anions; yet it shows no specific binding of the sort so characteristic of serum albumins. Moreover, as Dubos and Davis have shown, the binding is virtually abolished by heat denaturation of the albumin, although the positively charged groups remain. Some highly specific configurations in the native protein must evidently be required for the reaction.

\* I doubt whether the failure of  $\gamma$ -globulin to bind methyl orange can be explained by the pH and the isoelectric point of the protein. Klotz and Urquhart<sup>3</sup> carried out experiments both at pH 5.7, where the globulin is positively charged, and at 6.8, where it is almost isoelectric, and found no binding in either case. If there were binding at more alkaline pH values, this would be surprising, since the electrostatic effect of the negative charge on the protein would repel the anionic methyl orange, and make combination more unlikely than at an acid pH. Many anions are indeed bound by serum albumin, even when the protein is negatively charged, but the recent studies of Scatchard and his associates<sup>4</sup> using chloride and thiocyanate, show that binding of these anions increases as the net charge on the protein becomes more positive (or less negative), and the variation with protein charge can be satisfactorily explained by the Debye-Hückel theory.

As to van der Waals' forces, it seems to me they must play a role in such phenomena as the binding of fatty acid anions by serum albumin. Dr. Luck's extensive studies have shown that the binding becomes stronger for each additional carbon atom, in the series of anions from

\* In reply to Dr. Dervichian's remark, p. 19.

<sup>3</sup> *J. Amer. Chem. Soc.*, 1949, **71**, 1597.

<sup>4</sup> Scatchard, Scheinberg and Armstrong, Jr., *ibid.* (in press).

acetate to caprylate. Moreover, the binding is strong at concentrations of free anion far below those required for micelle formation. I should not care to generalize further. Many of the phenomena discussed at this meeting, by Dr. Dervichian and others, clearly require explanation along very different lines.

**Dr. D. G. Dervichian** (*Paris*) said: The experimental evidence<sup>17</sup> is that methyl orange binds less to  $\gamma$ -globulin than to serum albumin. Now these experiments have been done at pH 5.7, that is on the basic side of the isoelectric point for serum albumin and on the acid side for  $\gamma$ -globulin. Another experimental result<sup>14</sup> is that cationic detergents do precipitate with  $\gamma$ -globulin. It is obvious that specific differences exist between proteins, one of these differences being precisely the isoelectric point, and the most striking being the immunochemical specificity. These singularities are very probably bound to differences in the molecular structure and configuration (this being pure interpretation).

Dr. Edsall thinks that the contrast between serum albumin and  $\gamma$ -globulin in their possibilities of association is due to differences in the possibility of binding by van der Waals' forces and cannot be explained by simple ionic bonds. Now it is well known that the behaviour in the presence of ordinary electrolytes of albumins is also different from that of globulins (i.e. very different concentrations of  $(\text{NH}_4)_2\text{SO}_4$  are necessary to produce precipitation). Yet in this case it is obvious that the interaction is purely ionic. Similarly, if denaturation of proteins virtually abolishes the binding with lipids, it also modifies profoundly the interaction with ordinary electrolytes (e.g. precipitation). Yet no one would think of bringing in some highly specific configuration to explain the action of ordinary salts on proteins. No doubt the structure of proteins is highly specific, but it is not certain that this specificity of configuration intervenes in the interaction between proteins and ionic lipids. In fact, we were able to obtain phase separation (precipitation or coacervation) using indifferently either anionic and cationic detergents or electrolytes such as  $(\text{NH}_4)_2\text{SO}_4$ .

Finally, if by van der Waals' forces the bond were in specific relation to the nature of the lateral non-polar chains, it would be difficult to understand how, with a given protein, associations could be obtained with substances as different as fatty acids, phospholipids, nucleic acids, gum arabic, or dyes, whose paraffin chains are very different from the structural point of view.

**Prof. E. Chargaff** (*New York*) said: To me complexes between unspecified proteins and gum arabic, or even soaps, do not look exactly like my conception of lipo-proteins. In considering individual lipo-proteins we shall have to pay attention not only to the polar characteristics of the lipid and of the particular protein, but also to the structure of the latter. Obviously, serum albumin or globulin will not behave in the same manner as thymus histone towards lecithin or phosphatidyl serine. Simplification has, of course, its uses; but by carrying model experiments to such lengths, I am afraid, we may end up with the proverbial "knife without handle which has lost its blade".

**Dr. D. G. Dervichian** (*Paris*) said: Prof. Chargaff's remarks are highly instructive. They show how far force of habit and mere repetition of assertions often appear as argument even in scientific reasoning. From the fact that lipo-proteins are not dissociated in the electrical field, it has been concluded by Cohen and Chargaff<sup>18</sup> that the bond is not a simple saline bond. Now this conclusion is implicitly based on the very familiar behaviour of the very simple electrolytes in an electric field, where neither the anion ("blade") nor the cation ("handle") resemble the lipo-protein ("knife") constituents. Thus, while it seems very sound to compare a lipo-protein to NaCl, a colloidal electrolyte such as cetyl pyridinium bromide (which at least has a micellar cation) or even a protein-fatty acid mixture, are considered as models very remote from lipo-proteins.

It would be rather unfair to the authors of the twenty-six papers<sup>1-11</sup> from which general and consistent conclusions have been derived, to assert that their work refers to unspecified proteins. To say that natural lipo-proteins look exactly similar to the artificial mixtures of proteins and other colloidal electrolytes is certainly overlooking and overstating some of the conclusions of this paper.

The unquestionable fact which comes out from a detailed analysis of all the available work done on these associations in the course of the past forty years is that, whatever the protein (serum albumin, egg albumin, casein, edestin, haemoglobin, globulin, gelatin), or the other colloidal electrolyte (lecithin, fatty acids, anionic or cationic detergents, long-chain choline esters, bilirubin, nucleic acid, gum arabic, basic or acid dyes), the behaviour as function of the pH, salts present, and proportions, is the same.

It can be asserted, on the other hand, that : (a) as far as pH, action of salts and electrophoretic migration are concerned, natural lipo-proteins behave in a way parallel to that of the different artificial associations mentioned above ; (b) with regard to extraction and substitution of lipids, as well as the solubilization of the non-soluble lipids, are concerned, the behaviour of natural lipo-proteins recalls astonishingly the behaviour of artificial lipid-lipid associations. To ignore deliberately this generalization of facts would be to deny any interest in experimental results.

**Prof. M. Macheboeuf** (*Paris*) said : I should like to ask Dr. Dervichian how his very interesting theory takes into account the specificity possessed by lipidic cenapses ? I have been able to obtain from horse serum a cenapse which contains lecithin and esters of cholesterol only, whereas other lipids (non-esterified cholesterol, sphingomyelin, neutral fats) are found in other cenapses. How does Dr. Dervichian interpret this specificity, which seems to exclude certain lipid, although these are chemically closely related to those found in the cenapse ?

**Dr. D. G. Dervichian** (*Paris*) said : I can only make a tentative suggestion to explain these facts. We were able to show<sup>44, 45</sup> that, by associating lipids and similar substances, their affinity for water is strongly increased, sometimes reaching complete solubility under the form of mixed micelles. Thus, under their associated form, the lipids found in Prof. Macheboeuf's lipo-protein might show a greater affinity for water. Furthermore, their ionization might be increased by the ionic interaction of the protein molecules, thus producing complete solubilization. Therefore, depending on the conditions of precipitation (i.e. decrease of solubility by addition of salts or variation of pH) the less soluble lipidic associations could precipitate with certain proteins while the others remain in solution.

**Dr. G. Popjak** (*London*) said : It is easy to understand the postulated ionic lipid-protein association where the " lipid " is a soap, which readily dissociates, but does Dr. Dervichian think that in a lecithin molecule the esterified fatty acids dissociate ?

**Prof. D. G. Dervichian** (*Paris*) said : Certainly not—the ions to be considered in the case of lecithin are those of the phosphorylated choline radical.

**Prof. E. K. Rideal** (*London*) said : We have to thank Dr. Dervichian for a most interesting paper. As I understand the matter, Dr. Dervichian is so perturbed about the free energy change involved in a single molecule of lipid or detergent leaving its micelle and attaching itself to the protein by means of the polar group, leaving the non-polar group in the water, that he wishes to bring the whole micelle with him. I think we cannot give up the view that the non-polar portion of the detergent or lipid must take part in an interaction with part of the protein. This view is supported by penetration experiments, by drying, and by specificity of reaction, especially when we assume modification of structure of this portion of the molecule. We must remember that in reactions with

proteins it is frequently the protein carboxyl reaction which provides most of the energy.

**Dr. D. G. Dervichian** (*Paris*) said: In fact, account should be taken of the energy provided by the protein carboxyl reaction. The existence of the lipids under the micellar form has to be assumed whenever it is necessary to account for extra van der Waals' forces in the bond energy. This does not exclude the fact that single molecules would interact with the protein, as I have already admitted in my answer to Dr. Pankhurst. From my point of view, however, this interaction is again a charge neutralization in each element of volume, the two constituents remaining apart, and does not mean that the molecules of lipid stick on to the protein molecule.

## FORMATION OF LIPO-PROTEIN MONOLAYERS

### PART I.—PRELIMINARY INVESTIGATION ON THE ADSORPTION OF PROTEINS ON TO LIPID MONOLAYERS

BY P. DOTY \* AND J. H. SCHULMAN

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New techniques have been developed to follow the interactions of proteins with electrically charged monolayers at an air-water interface. When steric factors prevent the protein molecules from entering the monolayer, adsorption of the protein takes place, and this is shown to be dependent on the sign of the electrical charge of the compounds which meet at the interface. This adsorption is essentially reversible and accounts for the flocculation and re-dispersion of emulsions stabilized by anionic or cationic detergents, in contact with proteins at different pH's.

The kinetics of penetration and ejection of proteins in contact with charged interfaces have been studied at constant pressure. These processes are dependent on the electrical charge of the reactants and the surface pressure at which the experiments are carried out. Stoichiometric complexes are demonstrated between non-polar portions of the protein molecules and the long-chain ionic compounds, and the general forces, i.e. electrical forces, and van der Waals' forces involved in the association are reviewed.

Whereas adsorption follows a reversible pattern, monolayer expansion is essentially irreversible. The existence of "bound protein" at the charged interface is demonstrated and interpreted. The bearing of these results upon the interaction of proteins with long-chain ionic compounds in bulk solution, is considered.

Previous work<sup>1</sup> on protein monolayers suggests that radical changes of an irreversible nature take place on spreading of the protein at an air-water or oil-water interface. Force-area compression curves of the protein monolayers are rather irreproducible above certain surface pressures. This is due to the association of the reactive groups present in the large protein molecule. The forces involved in these associations are responsible for the gel state, or highly viscous characteristics of protein monolayers. For the same reasons, studies on monolayers obtained by spreading a solution containing protein, lipids or protein-soluble surface-active agents, are only of a qualitative nature, and

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<sup>1</sup> Cockbain and Schulman, *Trans. Faraday Soc.*, 1939, **35**, 1266.

show little bearing on the behaviour of proteins in aqueous solution in the presence of long-chain ionic compounds.

Injection of lipids or detergents under protein films leads to penetration or displacement of the protein, and the experimental results are again difficult to interpret quantitatively.<sup>2</sup> When non-surface-active agents, or poorly surface-active materials are injected underneath protein monolayers better results can be obtained. It has been shown that tanning agents possess a certain parallelism in their behaviour on protein films and on long-chain amines.<sup>3</sup>

To study the interaction of proteins with lipids, and detergents, it is necessary to avoid structural alterations of the protein which happen by spontaneous spreading and unfolding of the protein molecule at the interface. The protein is injected underneath the interacting monolayers maintained at a pressure equal or above 15 dynes/cm., and the reaction is followed in terms of the mechanical changes which occur through the injection. Such techniques lead to more quantitative information, and explain the behaviour of protein solutions in contact with charged oil-water interfaces. A direct examination of the forces involved in protein-detergent association is also obtained.

Further attempts are being made to study by this method immunity or pseudo-immunity reactions, such as certain lipids may undergo when associating with serum proteins. Two samples of this system are cardiolipin with luteic serum, and R.H. lipid with positive serum. For this purpose it was considered necessary to examine by surface techniques the interaction which insoluble lipid monolayers undergo with different serum proteins present in the underlying solution.

Part I demonstrates the adsorption on to lipid monolayers such as cardiolipin, cephalin, lecithin and cholesterol, of serum protein fractions. Part II analyses quantitatively the physicochemical conditions related to protein-lipid associations and establishes new types of lipo-protein complexes.

## Experimental

**Lipid Monolayers.**—It has been shown that protein monolayers on compression do not stand pressures greater than 16 dynes/cm. without crumbling or forming irreversible striations. Also in mixed films with cholesterol where weak association can be anticipated, the protein is ejected from the cholesterol monolayer into the underlying solution to form an adsorbed double layer at pressures equal to the collapse pressure of the protein.<sup>4</sup>

Therefore should the lipid monolayer be compressed initially to the collapse surface pressure of the protein and a very dilute protein solution be injected into the underlying solution a strong rise in surface pressure against time be noted, some association must have taken place between the lipid and the protein.<sup>4</sup> Care must be taken in comparing the magnitude of these surface-pressure increases with different lipids owing to the different compressibilities of the lipid films. Thus a cholesterol monolayer is condensed and very incompressible ( $40\text{--}39 \text{ \AA}^2$ ) and small decreases in area will give large changes in surface pressure. Whereas a cardiolipin film is liquid expanded and easily compressible over large areas ( $350 \text{ \AA}^2 - 185 \text{ \AA}^2$  per molecule), cephalin and lecithin are similarly easily compressible.

<sup>2</sup> (a) Neurath, *J. Physic. Chem.*, 1938, **42**, 39. (b) Bull, *J. Amer. Chem. Soc.*, 1945, **67**, 10.

<sup>3</sup> Cockbain and Schulman, *Trans. Faraday Soc.*, 1939, **35**, 716.

<sup>4</sup> Schulman and Rideal, *Proc. Roy. Soc. B*, 1937, **122**, 46.

**Cardiolipin Monolayers.**—Force-area curves of the cardiolipin monolayers on acetate buffer pH 5.1 reveal a liquid-expanded film compressible from  $350 \text{ \AA}^2$  to  $185 \text{ \AA}^2$  at a collapse pressure of 40 dynes/cm. This assumes a molecular weight<sup>5</sup> of 2200.

It is interesting that this molecular weight receives confirmation from the surface-film work. Six unsaturated fatty acid radicals are considered in the molecule. The limiting area per oleyl chain is thus  $31 \text{ \AA}^2$  which is nearly identical to the limiting compression area of a single chain of oleic acid. From the monolayers work it is easier to consider the structure as three dioleoyl glyceryl phosphoric acid ester units joined on one glycerine molecule and not as described by Pangborn<sup>5</sup> with the oleic acid radicals separated by the phosphoric acid and glycerine units in the ratio 2/1/1/2. Force-area curves of the cardiolipin over the whole pH range would possibly clarify this point.\*

**Protein Solutions.**—The quantity of protein injected into the underlying buffered aqueous solutions was so chosen that its rate of adsorption on the free aqueous side of the Langmuir trough was very small over the time period of the reaction with the lipid monolayer. Before each reading on the torsion head of the Langmuir balance, this free side of the trough was cleaned by waxed slides. In Part II where the automatic pressure measuring device was used this technique was not required since the vertical pressure on the hydrophilic plate hanging in the free side of the trough compensated for the surface pressure of the adsorbing protein (see Part II).

A very convenient concentration of the protein in the underlying solution was found to be 2 mg. for 300 ml. Various serum protein fractions were obtained by ammonium sulphate precipitation and dialysis or standard protein fractions.† Haemoglobin was obtained from laked red cells and purified by centrifuging in NaCl and dialyzing. In analysing the results the possible presence of lipids in the protein fractions was taken into consideration.

**Procedure.**—The lipid monolayer is compressed to 14 dynes/cm. on a Langmuir trough and the equivalent of 2 mg. protein in 10 ml. solution, injected into the underlying solution and vigorously circulated. The increase in surface pressure of the lipid film with time, is noted at constant area.

In Part II the gradual expansion (penetration) of the lipid film at constant pressure by injection of the lipid protein solution is noted with time. Fig. 1-3 shows the changes in surface pressure of the negatively charged lipid monolayer cardiolipin with time in the presence of serum protein fractions, starting at surface pressures above the collapse pressure of the protein films alone. These curves show that on the acid side of the isoelectric points of the various protein fractions strong association takes place with the negatively charged lipid film.

On the alkaline side, strong inhibition of this effect takes place. The slow rise in surface pressure that is observed on the alkaline side is possibly due to two causes (i) a general non-specific surface solution of the protein into lipid monolayer (see Part II) and (ii) serum lipids associated with the injected protein fraction can preferentially associate with either the film-forming lipid or the protein in solution. This is most noticeable

<sup>5</sup> Pangborn, *J. Biol. Chem.*, 1944, **153**, 343; 1947, **168**, 358.

\* See Grazer, This Discussion.

† Protein fractions were supplied by Prof. John Edsall and were prepared from blood collected by the American Red Cross under contract between the Committee on Medical Research of the office of Scientific Research and Development and Harvard University. Protein fractions were also kindly supplied by Armour Co. Ltd.

We are grateful to Dr. Mary C. Pangborn for help in obtaining information and supplies of cardiolipin, and to Prof. Blix, Uppsala, Sweden, for cephalin samples.



in the case of  $\alpha$ -globulin which is known to have surface-active lipoids associated in this fraction ; whereas the albumin and  $\gamma$ -globulin fractions do not show this phenomenon.

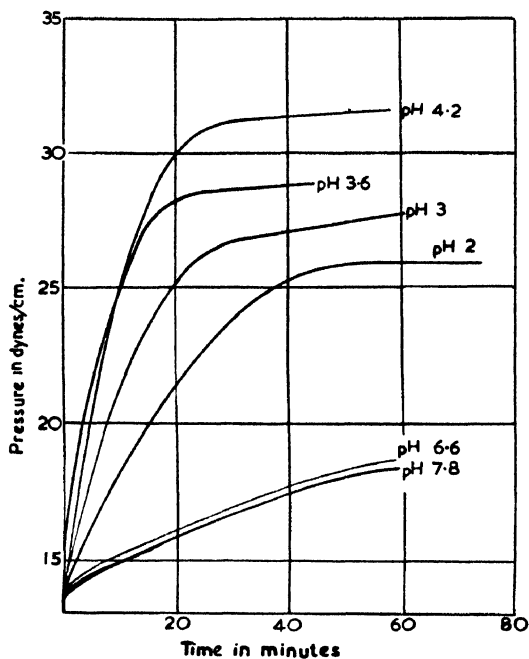


FIG. 1—Pressure rise of cardiolipin monolayer on injection of human albumin, pH effect.

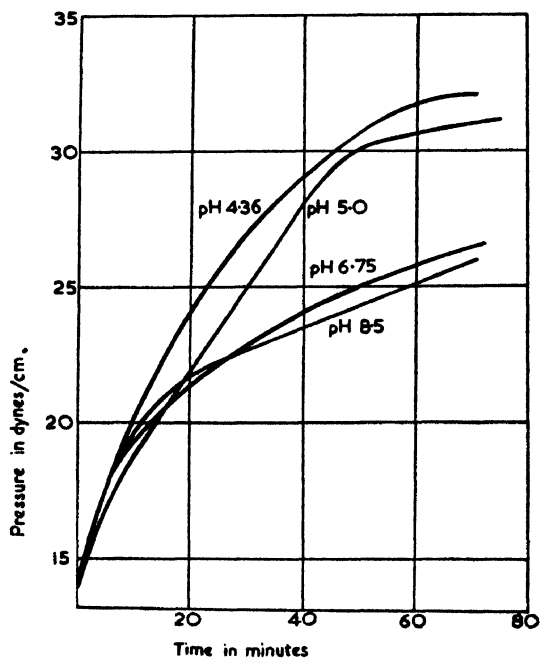


FIG. 2—Injection of  $\alpha$  globulin, pH effect.

In Fig. 5 a curve is taken from the work of Czczowiczka<sup>6</sup> using whole horse serum protein injected under a cholesterol film. This demonstrates the competitive lipid-protein effect in solution, since with a cholesterol monolayer reacting with albumin no pH effects are observed. Thus with a lipid-free protein the surface solution of protein molecules into uncharged

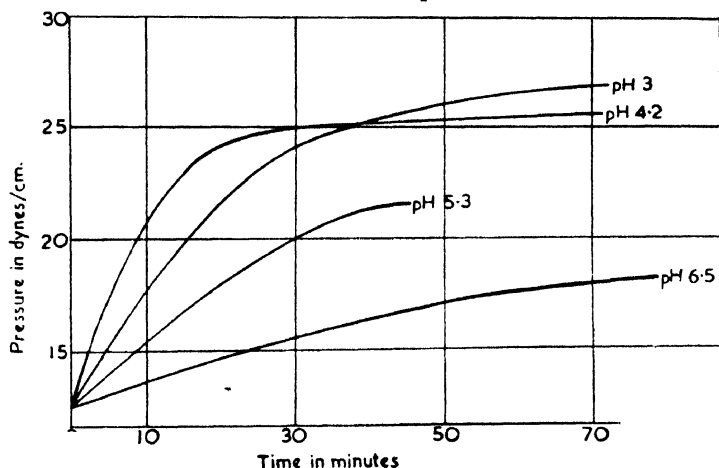


FIG. 3—Pressure rise in cardiolipin monolayer on injection of  $\gamma$  globulin, pH effect.

lipid monolayer, such as cholesterol, is small and independent of the pH. In Fig. 5 the rise in surface pressure of a cholesterol film is seen to be comparable with the surface-pressure rises in reacting charged lipid-protein systems. This is shown in Part II to be quite small when the incompressibility of the cholesterol film is taken into account. On analysis

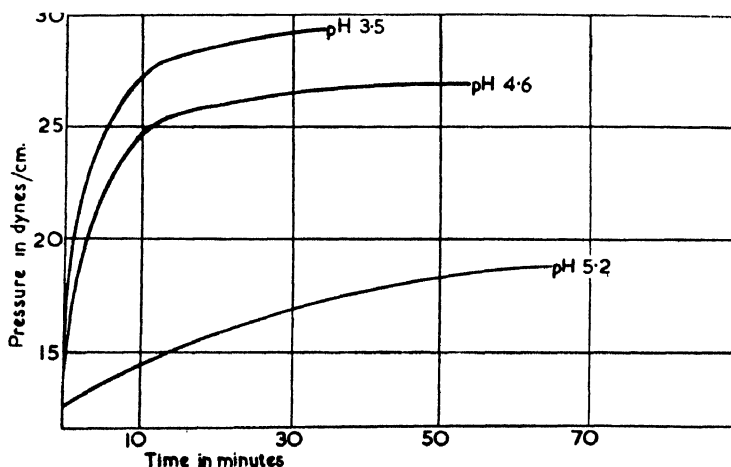


FIG. 4—Pressure rise of cephalin monolayer on injection of human albumin, pH effect

of the curve obtained by expansion at constant pressure, it is found that simple surface solution in the cholesterol monolayer is taking place.

Fig. 4 shows albumin associating with a cephalin film which behaves as a negatively charged monolayer over pH range 2-14. The albumin-cephalin association cuts out sharply at the isoelectric part of the albumin.

<sup>6</sup> Schulman, *Biochem. J.*, 1945, **39**, 54.

No association takes place at pH's more alkaline than pH 4.6. No association could be measured with the serum protein fractions with lecithin monolayers over the pH range 3-11.

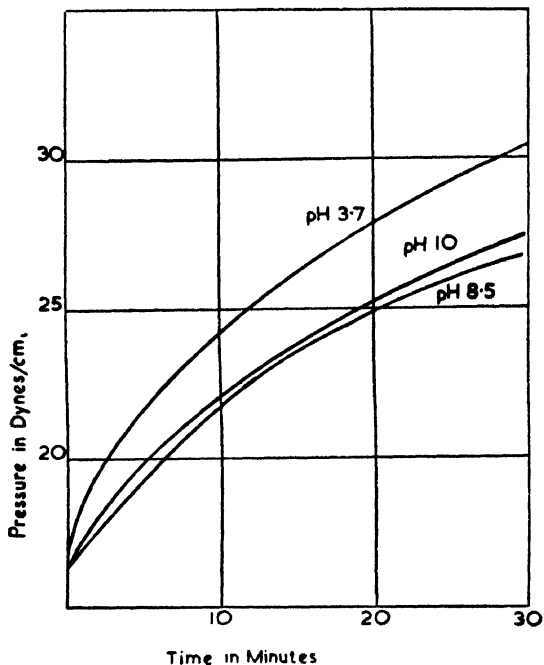


FIG. 5—Pressure rise of cholesterol monolayer on injection of horse serum protein (1), pH effect.

**Cardiolipin—Luetic Sera.**—Attempts were made to measure by this technique the possible specific association of luetic sera and mixed films of cardiolipin-lecithin and cholesterol in varying proportions. No association could be measured other than that given by the normal sera.

This was surprising in view of the fact that in the Kahn reaction, the specific adsorption and isolation of a globulin protein has been established on the mixed suspensions of the three above-mentioned lipids in the presence of luetic serum.<sup>7</sup> Globulin fractions obtained from the luetic sera also gave no positive association on the mixed lipid monolayers.

A possible explanation could be that in these surface technique experiments, the concentration of the protein in solution is about 1/200,000 whereas in the Kahn reaction association of the globulin on the mixed lipid suspension in a very strong positive luetic serum rarely exceeds a dilution of the luetic serum of more than 1/100 (on protein about 1/1200). It is not possible at present by surface techniques to work at these high protein concentrations. It might be possible to measure these types of specific lipo-protein associations by surface techniques in those systems (RH lipid) where the protein is reactive in very dilute solutions.<sup>8</sup>

**Analogy with the pH-controlled Emulsion Flocculation Work.**—

The preceding result is even more surprising when one considers the analogy of the flocculation of emulsions of oil droplets<sup>9</sup> with the pH-controlled monolayer adsorption work described in this paper. Similar long-chain ionic compounds are used in both works in the presence of protein solu-

<sup>7</sup> Eagle, *Lab. diag. Syph.* (St. Louis, 1937).

<sup>8</sup> Price, *J. Amer. Chem. Soc.*, 1948, **70**, 3527.

<sup>9</sup> Elkes, Frazer, Schulman and Stewart, *Proc. Roy. Soc. A*, 1945, **184**, 104.

tions in concentration sufficient to cover the surface of the emulsion droplets with a monolayer of proteins.

In the Kahn reaction the protein can be adsorbed specifically against the charge on the aggregate, since the specific adsorption functions equally well in acid or alkaline solution. The cardiolipin-*lecithin* surface is negative and the protein also can be negative on the alkaline side of its isoelectric point. The repulsion forces thus arising are not sufficient to prevent specific adsorption of the protein molecules in the luetic serum. It could then be considered that specific action of the luetic protein is related to the non-polar portion of the protein molecule.

In Part II where the irreversible penetration of the protein molecule or bound protein is considered on the alkaline range of the isoelectric point for negatively charged lipid monolayers in contrast to the reversible adsorption of the adsorbed protein molecules, an analogy with, or interpretation of the Kahn reaction may be found.

Our thanks are due to Dr. J. H. McCoy for considerable help and encouragement and to Mrs. M. Doty for collaboration in the experimental work.

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## PART II.—MECHANISM OF ADSORPTION, SOLUTION AND PENETRATION

By R. MATALON \* AND J. H. SCHULMAN

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Most of the work on the interaction of proteins with long chain ionogenic compounds such as detergents has been carried out in bulk solution.<sup>1</sup> When studying these interactions at interfaces two main difficulties are encountered. They are (i) spontaneous spreading and unfolding of the molecule when a protein spreads at pressures less than 15 dynes/cm.<sup>2</sup>, (ii) the marked solubility of the detergents in water which prevents the formation of stable monolayers at the air-water interface.<sup>3</sup> To obviate these difficulties long-chain ionic compounds of a pronounced hydrophobic character were spread at the surface, kept at pressures equal to or above 15 dynes/cm. and the proteins were then injected into the underlying solution. These long-chain ionic compounds are structurally analogous to the usual detergents in that they contain a polar group and a hydrophobic tail; the only difference is the increased length of the hydrophobic residue of the molecule which is responsible for the marked stability of the monolayers on the surface of the water.

Cardiolipin and  $C_{22}H_{45}SO_4Na$  produce negatively charged monolayers at all pH values. Stearylcholine on the other hand gives

\* Oliver Gatty student.

<sup>1</sup> Putnam, *Advances in Protein Chemistry*, 1948, 4, 79.

<sup>2</sup> Cockbain and Schulman, *Trans. Faraday Soc.*, 1939, 35, 1266.

<sup>3</sup> Bull, *J. Amer. Chem. Soc.*, 1945, 67, 10.

positively charged monolayers provided that care is taken to exclude polyvalent anions such as phosphate,<sup>4, 5</sup> which, on adsorption on to the monolayer, can discharge it and possibly reverse its sign. The behaviour of uncharged monolayers has also been studied and, in these instances, cholesterol has been used.

On studying the interaction of these monolayers with proteins, it is possible to distinguish three processes: adsorption, penetration, and solubility.

Adsorption is observed under certain conditions where the protein is injected below a monolayer kept at constant area. The protein is thus prevented from entering extensively into the surface, and the pressure rise observed is mainly due to the association of the polar groups of the protein with those of the monolayer.

Similarly, solution and penetration of the protein take place when the pressure of the monolayer is kept constant. During these latter processes, spontaneous expansion of the monolayer takes place. The rate of extension determines whether the protein is entering the surface by solution in the monolayer or by molecular interaction resulting in the association of the polar groups of the reactant, and van der Waals' attraction between the long hydrocarbon residue of the monolayer and the side chains of the proteins.

### Experimental

New techniques developed<sup>6</sup> recently for studying monolayer interactions at air-water interfaces have been applied to this particular study. Although monolayer interactions have been the subject of a great deal of work one practical difficulty is inherent in this method when the reactant injected is surface active. In these conditions, the pressure measured is no longer that of the interacting monolayer, but is the pressure difference between this monolayer and the pressure of the film of the adsorbed solute on the free water side of the boom. Hitherto the surface pressure value was obtained by repeatedly sweeping the free water side with waxed slides to remove the adsorbed molecules. As well as disturbing the surface, a certain error was inevitable as the adsorbed layer was never completely removed by this process. The technique adopted in these experiments introduces a device<sup>6</sup> which automatically cancels the pressure of the adsorbed layer and so renders the cleaning of the surface unnecessary. The principle of the compensation is that a monolayer exerts upon a surface passing vertically through it an upward pressure equal to that which it exerts horizontally. Hence a hydrophilic plate dipping in the free water side can be so attached to the torsion wire that the moments of the horizontal and vertical forces about the centre of the wire are opposite and equal.

Furthermore the general technique frequently used for studying the phenomenon of penetration was the recording of the compression curve of the mixed film. The existence of stoichiometric complexes was deduced from the changes of slope, or the kinks, of the compression curve. Should the compression be carried out at a rate greater than the rate of ejection of the solute injected, crumpling of the monolayer occurs thus leading to metastable states<sup>7</sup> difficult to interpret. This has been shown to be most pronounced with rigid or solid interacting monolayers.

To avoid these complications, the technique of spontaneous extension

<sup>4</sup> Schulman and Cockbain, *Trans. Faraday Soc.*, 1940, **35**, 663.

<sup>5</sup> Elkes, Frazer, Schulman and Stewart, *Proc. Roy. Soc. A*, 1945, **184**, 104

<sup>6</sup> Matalon and Schulman, *J. Colloid Sci.*, 1949, **4**, 89.

<sup>7</sup> Matalon and Schulman, *Trans. Faraday Soc.*, 1947, **43**, 479.

or ejection of the monolayer at constant pressure, first used by Schulman and Stenhagen,<sup>8</sup> has been developed.<sup>9</sup>

The Langmuir trough to which is attached the compensating plate is equipped with a constant pressure device which operates a relay. This relay controls the movement of a motor which expands or compresses the monolayer when the surface pressure acting on the boom is above or below a certain value. This value can be adjusted before setting the experiment. By using a platinum-mercury contact the pressure can be maintained constant within 0.2-0.4 dyne, and the surface variation of the monolayer under injection can be followed with great accuracy.

**Adsorption and Desorption at Constant Area.**—The protein dissolved in a buffered solution is injected into the bath which is on the positive side of the isoelectric point when the surface is covered by negatively charged monolayers such as cardiolipin or  $C_{22}H_{45}SO_4Na$ . For stearylcholine, a positively charged monolayer, the pH at which the protein is injected is 10 and an acetate buffer is present in the bath.

The presence of the buffer permitted small and gradual variation of the pH values in the buffering range of the salts: this insured a constant pH after an injection during the course of the experiments.

## Results

The rise in pressure of the monolayer kept at constant area following this injection is recorded with time until a state of equilibrium is reached.

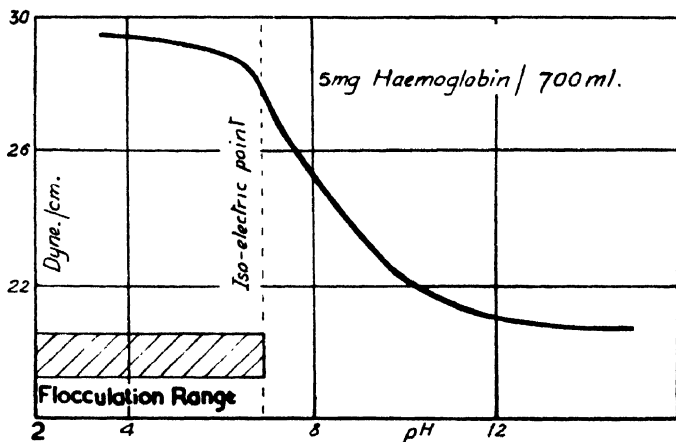


FIG. 1—Reversible adsorption of sheep haemoglobin on to cardiolipin. (Negative interface).

This is usually attained 40 min. after the injection of the protein. When the levelling of the pressure-time curve is reached small amounts of alkali or acid are injected into the trough, and the pressure variation of the surface film is recorded in parallel with the pH variation. The pH is measured using indicators, with an accuracy of 0.2-0.3 pH unit in the pH range 3.6 to 10.

(a) **PROTEIN AND MONOLAYER OPPOSITELY CHARGED** (Fig. 1-5).—When the protein is injected so that the sign of its electrical charge is reverse of that of the monolayer, the pressures reached vary between 27 and 30 dynes/cm., except in the case of  $C_{22}H_{45}SO_4Na$  and albumin where this pressure is about 37-38 dynes/cm. The marked increase in pressure observed in this particular system is due to the very low compressibility of the  $C_{22}H_{45}SO_4Na$  monolayer, and to the marked length

<sup>8</sup> Schulman and Stenhagen, *Proc. Roy. Soc. B*, 1938, 126, 356.

<sup>9</sup> R. Matalon (unpublished work).

of the hydrophobic portion of this molecule which increases the affinity to the few side chains of the polypeptide backbone of the protein which can enter into the monolayer.

(b) PROTEIN AND MONOLAYER SIMILARLY CHARGED (Fig. 1-5).—As soon as the pH is altered so that the sign of the protein becomes the same

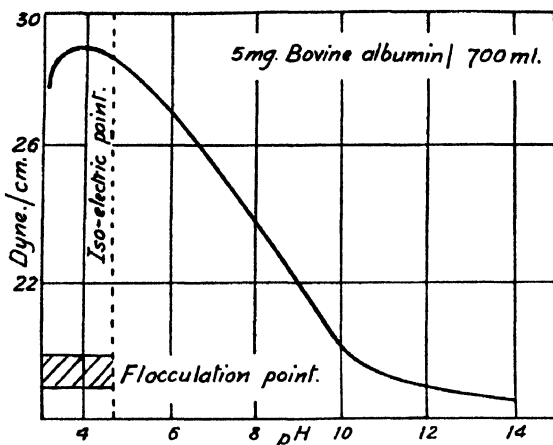


FIG. 2—Reversible adsorption of bovine albumin on to cardiolipin. (Negative interface).

as that of the monolayers, a decrease in the pressure is observed. Desorption is quite rapid and is over in a period of 15 to 20 min.

The essential feature of these experiments is their reversibility, in that

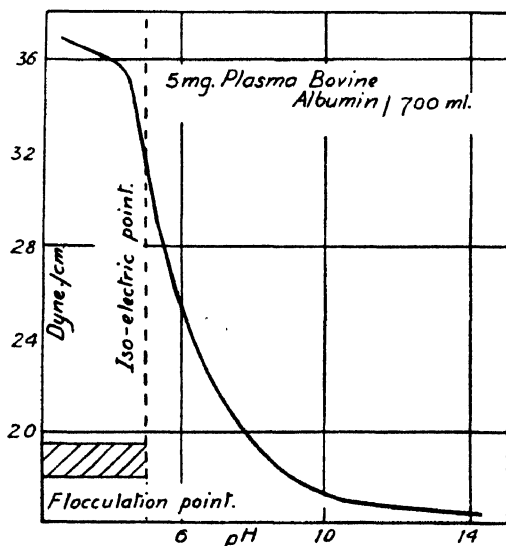


FIG. 3—Reversible adsorption of bovine albumin on to  $C_{12}$  sulphate. (Negative interface).

the pressure of the monolayer can be raised to its initial value, after desorption, by adjusting the pH to its original value.

(c) ANOMALOUS BEHAVIOUR OF  $\gamma$ -GLOBULIN.—In the case of  $\gamma$ -globulin this reversibility is not so marked: 0.6 % of NaCl is used in the trough to obtain the protein in solution.

When the  $\gamma$ -globulin-cardiolipin system has been slowly brought from pH 4 to pH 10, to construct curve 1 of Fig. 4 and when it is restored to the original pH, a pressure sensibly higher than the original is found. If it is now again brought slowly over the pH range studied in curve 1, the same shaped curve is produced (curve 2) with this increase of pressure maintained throughout.

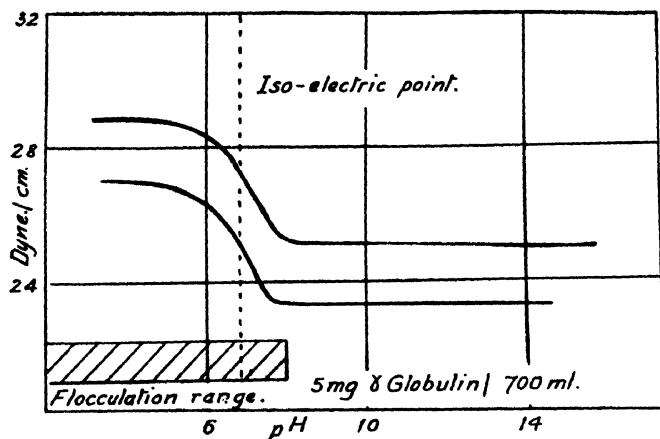


FIG. 4—Adsorption of  $\gamma$ -globulin (0.6% NaCl) on to cardiolipin. (Negative interface).

**General Behaviour of the Desorption Curves.**—Although by acid or alkali injections the sign of the protein can be reversed as the isoelectric point is crossed, the entire pressure variation is not completed at the reversal of the charge (Fig. 1-5), but occurs gradually upon varying the pH and reaches a constant minimum value. These values are generally greater than 15 dynes/cm. The actual figures recorded are :

System		Pressure Dynes/cm.
Protein	Monolayer	
Bovine albumin	Cardiolipin	17.5
	$C_{22}H_{45}SO_4Na$	18.5
Haemoglobin	Cardiolipin	21
	Stearylcholine	21
$\gamma$ -Globulin	Cardiolipin	24.3 (curve 1)
		25.5 (curve 2)

The original pressure of the monolayer is 15 dynes/cm. From these results it appears that the pressure of the monolayer attained by adjusting the pH of the bath to the non-reactive protein, is essentially dependent on the nature of the protein and does not seem related to the nature of the monolayer present at the interface.

While with bovine albumin on cardiolipin or  $C_{22}H_{45}SO_4Na$  a pressure rise of 2.5-3.5 dynes/cm. above the initial pressure of the monolayer is observed; with haemoglobin on cardiolipin or on stearylcholine this pressure difference is 6 dynes/cm.

The marked increase of 9.3-10.5 dyne/cm. observed in the case of  $\gamma$ -globulin with cardiolipin is probably due to the high molecular weight of this protein and also, possibly, to the existence of traces of lipids present as an impurity in the fraction under investigation.



**Solution and Penetration at Constant Pressure.**—In order to determine the influence of electrical forces on the association of protein with long-chain ionic compounds, the behaviour of a neutral molecule such as cholesterol, in the presence of proteins has been studied and compared with the association of negatively charged monolayers with proteins.

(a) **SOLUTION EFFECT.**—**HAEMOGLOBIN-CHOLESTEROL.**—When haemoglobin is injected at pH 4.6-4.8 under a cholesterol monolayer kept at

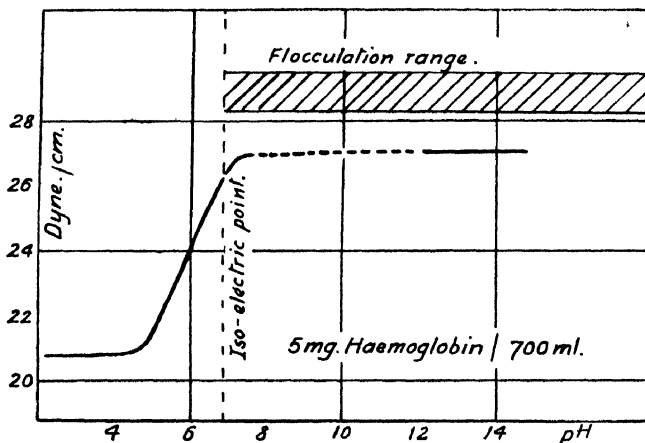


FIG. 5—Reversible adsorption of sheep haemoglobin on to stearyl-choline. (Positive interface).

14 dynes/cm. pressure an expansion is observed (Fig. 6) which after 7 min. becomes linear. This indicates that the expansion of the cholesterol is due to solution of the protein in the monolayer and not to a specific interaction between the two compounds. This conclusion is supported by the previous work<sup>9</sup> where gliadin is ejected from a cholesterol monolayer at its own collapse pressure.

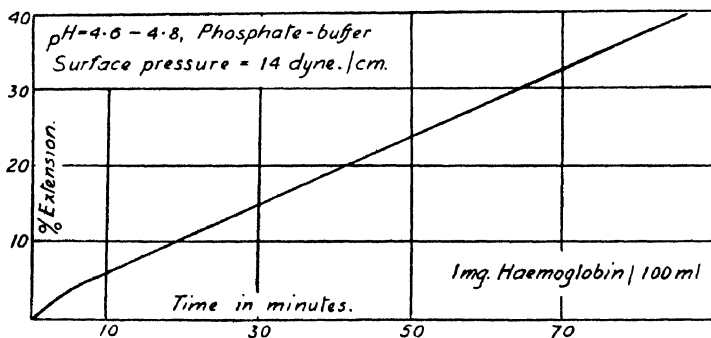


FIG. 6—Surface solution of haemoglobin in a cholesterol monolayer.

Seventy minutes after the injection of the haemoglobin the cholesterol monolayer had extended by 33 % but the film was still liquid, while on the free water side was a strong gel due to the formation of an adsorbed layer of the protein.

(b) **SOLUTION EFFECT AND PENETRATION.**—(i) *Protein and monolayers oppositely charged.*—Haemoglobin injected under a film of cardiolipin at 25 dynes/cm. pressure and at pH 4 causes rapid extension of the monolayer followed, after 55 min. by a linear expansion similar to the solution effect observed on the cholesterol monolayer. Thus the curve

obtained (Fig. 7) is a summation of rapid penetration and slow solution, the two parts of which can be distinguished by projecting the linear portion of the curve back to the extension axis. The area at which this line cuts the axis is that increase due to complete saturation of the monolayer by penetration, the remaining increase is due to the process of solution.

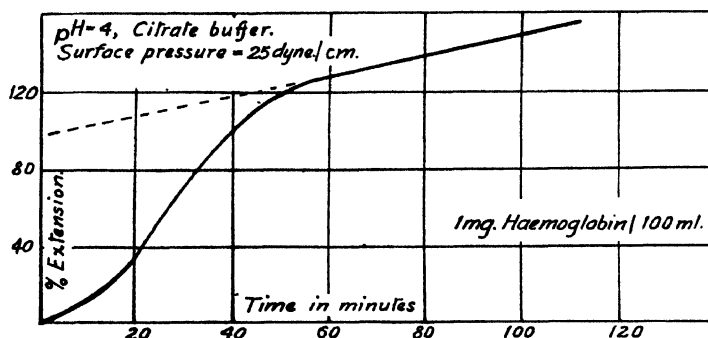


FIG. 7.—Interaction leading to complexes between haemoglobin and cardiolipin.

(ii) *Neutral protein and negative interfaces.*—If haemoglobin is now injected at its isoelectric point, no expansion is observed due to protein entering the  $C_{22}H_{45}SO_4Na$  monolayer kept in a pressure range of 28 to 20 dynes/cm., for a period of 35 min. If, after this period, acid is injected to shift the pH to 1, well on the acid side of the isoelectric point, very strong penetration occurs at 28 dynes/cm. pressure, and 100% extension

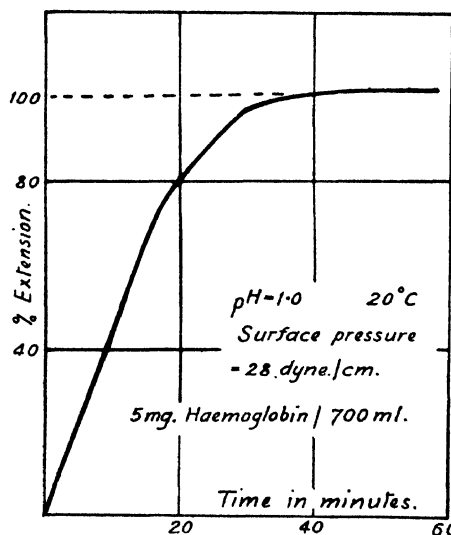


FIG. 8.—Interaction leading to complexes between haemoglobin and  $C_{22}$  sulphate.

of the original area is produced within 40 min. This shows that the phenomenon of penetration is controlled by electrical forces.

(iii) *Physical state of expanded monolayers.*—While the cholesterol monolayer remained liquid after the expansion, cardiolipin and  $C_{22}H_{45}SO_4Na$  become strong gels when the protein penetrates them.

(iv) *Influence of steric factors on penetration.*—When the haemoglobin is injected at pH 3 below a  $C_{22}H_{45}SO_4Na$  monolayer at 31 dynes/cm.

pressure, the maximum extension observed is 33 % (Fig. 9, curve Ia). A further extension to 66 % is obtained by adjusting the pressure to 28 dynes/cm. (Fig. 9, curve Ib). Finally at 25 dynes/cm. the monolayer soon

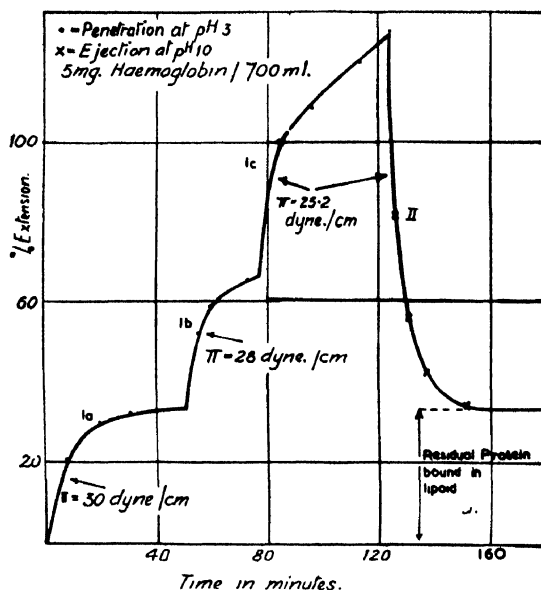


FIG. 9.—Surface pressure and pH variation on the kinetics of penetration and ejection of haemoglobin with  $C_{28}$  sulphate. The existence of "bound protein."

develops a constant linear increase showing an added solution effect (Fig. 9, curve Ic). These experiments demonstrate that the closer the packing of the chains in the monolayer the less penetration occurs.

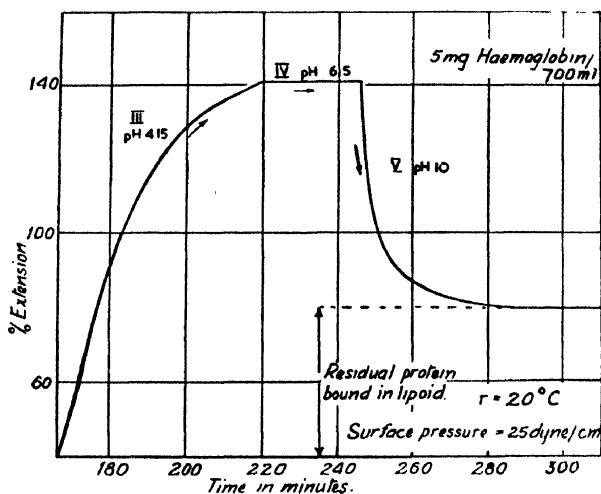


FIG. 10.—Demonstration of irreversibly "bound protein" in system  $C_{28}$  sulphate-haemoglobin.

(v) *Existence of "Bound Protein."*—If under this haemoglobin  $C_{28}H_{48}SO_4Na$  system at pH 3, NaOH is injected to increase the pH to 10, rapid ejection of the protein takes place from the monolayer (Fig. 9,

curve II) and the area attained once the ejection is complete exceeds the original area of the unpenetrated monolayer by 35 %. This increase in area is due to irreversibly "bound protein" associated with the interface by non-electrical forces, while the protein readily ejected by pH changes is associated with the monolayer mainly by the action of electrical forces and is thus expelled when the sign of the protein is reversed.

Re-expansion of the monolayer can readily be obtained if the pH is now decreased to 4.2 by a new injection of acid. In this case the complex protein  $C_{22}H_{45}SO_4Na$  is reformed but the rate of solubility of the protein in the monolayer has increased, i.e. the slope of the linear part of the curve is steeper (Fig. 10, curve III). It is interesting to note that if the pH is again increased to 6.4-6.5 close to the isoelectric point, no displacement of the interface is observed for a period of 11 min. and the area is constant (Fig. 10, curve IV).

Re-ejection of the protein is now obtained on altering the pH to 10. When equilibrium is reached the monolayer occupies an area 80 % greater than its original area before penetration (Fig. 10, curve V).

Although penetration of a charged monolayer does not take place at the isoelectric point, once a monolayer is penetrated, only reversal of the sign of the protein will eject it. Furthermore, reversing the sign of the protein causes ejection of some of the protein only.

### Discussion

**Adsorption and Desorption.**—Desorption of proteins from electrically charged monolayers has been studied by following the variation of

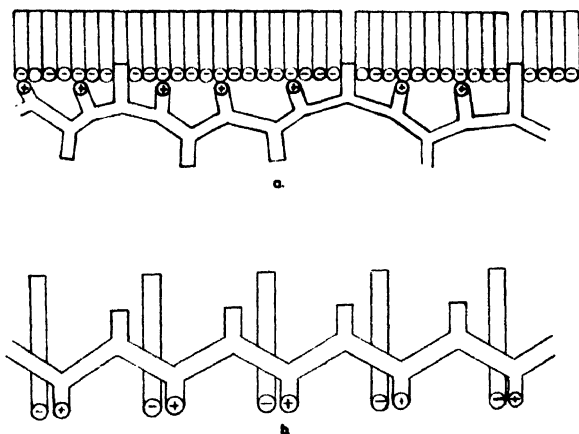


FIG. 11.—Structure of the mixed films: protein—negatively charged monolayer.  
a. on reversible adsorption. b. on irreversible penetration.

surface pressure with varying pH on either side of the isoelectric point (Fig. 1-5).

The experiments carried out at constant area show the mechanism of one type of association between the protein and the charged interface, but a more complete interpretation of the results is obtained when the expansion experiments are considered at the same time.

A marked rise in surface pressure follows injection of proteins under electrically charged monolayers kept at constant area, if the protein and the monolayer are oppositely charged. This rise is almost entirely due to polar-polar interaction, van der Waals' forces are not appreciably involved since as expansion experiments show, the protein cannot enter the film at the pressures thus reached (Fig. 11a).

It might be expected that the polar-polar interaction between the monolayer and the protein will increase as the pH becomes more distant from the isoelectric point, as it is known that the charge of the protein increases the further the pH is moved from this point. But experimentally the pressures observed on the desorption curve are almost constant in the whole range of pH where protein and spread monolayer are oppositely charged; this is because the structure of the protein-monomer association limits the possible rise. Indeed, the maximum pressures at which marked expansion of monolayers by proteins can occur is only slightly less than the pressures at which these penetrated monolayers are ejected and collapsed (33 dynes/cm.). For example if haemoglobin is injected under cardiolipin at pH 4 and the surface pressure maintained at 25 dynes/cm. (5 dynes/cm. lower than the pressure observed 36 min. after the injection (30 dynes/cm.) for this particular system at constant area) a rapid extension of the monolayer will occur, doubling its area in 36 min. (Fig. 7).

Furthermore, the maximum pressures that such a penetrated film can sustain, can be determined by recompression of the extended film to its original area. This pressure is found to be 33 dynes/cm. which is 8 dynes/cm. higher than the expansion pressure (25 dynes/cm.) and 3 dynes/cm. greater than the maximum pressure (30 dynes/cm.) attained at constant area. It is thus impossible to follow the varying degree of affinity of the protein and monolayer when oppositely charged, solely by studying the desorption curve.

The expansion technique would enable these variations to be studied. No expansion of a  $C_{22}H_{45}SO_4Na$  monolayer by haemoglobin takes place at the isoelectric point at 25 dynes/cm. pressure, while a very rapid expansion occurs when the protein is well on the acid side. Furthermore, if expansion is done at 28 dynes/cm. at pH 1, 100 % area increase is observed (Fig. 8), while at pH 4 66 % only of the extension is produced (Fig. 9, curve 1b).

Considering again the desorption curve in the pH range where the sign of the protein becomes identical with that of the monolayer, as the protein increases in charge, the pressure falls in all systems investigated. But this fall is gradual, and is complete only at pH values well separated from the isoelectric point (Fig. 1-5).

Again it would have been expected from the amphoteric character of proteins, and from the mechanism of the adsorption process, mainly polar-polar interaction, that the surface pressure would have suddenly decreased to the original pressure of the monolayer (15 dynes/cm.) on crossing the isoelectric point.

These experimental results indicate that desorption of the proteins involves other factors than the overcoming of electrical forces. We know that the rise in pressure observed is due to some compression of the monolayer by the few side chains of the proteins which enter it, and which associate by van der Waals' forces to the long hydrophobic chains present at the surface. Hence complete desorption will necessitate overcoming these forces also.

In fact, even when maximum desorption is reached the pressure of the monolayer is still higher than its original pressure: the greater the molecular weight of the protein, and the higher the probability of the existence of uncharged lipids, the higher is the pressure reached at complete desorption.

The rise in pressure above 15 dynes/cm., of charged monolayers

by proteins similarly charged is not only observed in the desorption process but in the adsorption process (see part I). The mechanism of this rise may be found in the solution effect. The pressure rise obtained when the protein and monolayer are similarly charged does not mean that there is strong association between them under these conditions. In fact, detailed analysis will show that this expresses only very few contacts.

The important point is that as the isoelectric point is crossed the pressure falls. Now the pressure reached at maximum desorption indicates a saturation in the adsorbed layer and this saturation has been demonstrated by the expansion technique. Indeed, at acid pH values the protein is extensively accumulated below the negatively charged monolayer and orientated by an attractive electrical field of force. This is shown by the marked increase in area of the monolayer when the protein is allowed to enter the surface. It can be shown too, that at and beyond the isoelectric point no penetration occurs, i.e. throughout the region where the protein and monolayer are similarly charged.

**Solution and Penetration.**—Expansion experiments show that solution and penetration of proteins take place during the extension of monolayers, and it is easy to distinguish the two processes by studying the rate of extension. Solution effect is due to simple diffusion of the protein to the surface and this process is characterized by a constant rate; thus a linear extension is obtained (Fig. 6).

Penetration is due to a marked interaction between the monolayer and the protein in solution. The rate of this interaction is therefore dependent on the concentration of the two reagents. For a constant concentration of the protein in solution the rate is proportional to the number of non-associated molecules present in the surface. Thus penetration follows a law of pseudo first-order reaction (since the concentration of the reacting protein is not varied by the reaction), and the rate of extension decreases as the monolayer expands.

It is experimentally possible to show the existence of the solution effect exclusive of any other process, whilst penetration is in most cases accompanied by solution. Failure to distinguish between the two processes had led several authors dealing with the penetration phenomena to doubtful conclusions as to the conditions of existence of stoichiometric<sup>10</sup> complexes. In the case of haemoglobin interacting with cardiolipin (Fig. 7) or  $C_{22}H_{45}SO_4Na$  (Fig. 8), 100% extension due to penetration was shown to exist. This indicates that one chain of the monolayer associates with one chain of the protein (Fig. 11b). It is therefore possible to characterize stoichiometric complexes which result from "chain interaction."

This result is explained if the protein has available, side chains orientated towards the monolayer. It appears therefore that the globular structure of proteins in solution with boundaries covered with hydrophilic groups only, is being altered when in contact with long-chain ionic compounds. Such an alteration would help to explain some of the effect of detergents on the structure of proteins. Whereas in the adsorption of proteins on to charged interfaces van der Waals' forces are of a minor importance, it would appear that these forces are responsible for the expansion of uncharged monolayers such as cholesterol.

<sup>10</sup> Schulman, Stenhagen and Rideal, *Nature*, 1938, 141, 785. Joly, *Nature*, 1946, 158, 26.

**"Bound Protein."**—The effect of pH on the expansion of charged interfaces shows the effect of electrical forces, while the irreversibility of the expansion demonstrates the van der Waals' forces. Indeed it has been shown that expansion of a charged monolayer does not take place at the isoelectric point, and that the monolayer already expanded at convenient pH values does not contract when the pH is adjusted to the neutral region of the protein.

Extra energy is needed to eject from the surface the side chains of the proteins associated by non-polar forces to the monolayer and this energy can be supplied by reversing the charge of the protein. In this case only partial ejection occurs.

The "bound protein" is a constituent of the expanded monolayer which cannot be ejected by altering the pH. The nature of this "bound protein" is of great interest. Although more experiments are still needed, it would appear that the bound protein is that which has entered the surface by a mechanism which involves only the action of van der Waals' forces and which is completely independent of any polar-polar interaction. Such a mechanism is found in the solution effect and has been proved to take place simultaneously with the penetration.

The probable structures of adsorbed proteins on to charged monolayers and of the chain complexes between protein and long-chain ionic compounds as obtained by penetration have been described in Fig. 11.

**Analogies to Reaction in Bulk.**—Previous work shows cases of reversible and irreversible changes when the proteins are adsorbed on to charged oil-water interfaces, as with emulsions, and then desorbed. Haemoglobin for example is regenerated as parahaematin<sup>5</sup> which is less soluble, and has lost its biological properties, whereas snake venoms and toxins can regain their full activity on desorption.<sup>11</sup>

The present work has differentiated two mechanisms of interaction of proteins at charged interfaces. Adsorption which is readily reversible involves mainly interaction of polar groups which are available on the surface of the protein molecule without involving any change of the molecular configuration, whereas penetration necessitates radical alterations in the molecular structure to enable the non-polar chains to enter into the surface, and associate by penetration with the non-polar portion of the film forming molecule.

By analogy it could thus be assumed that where irreversible changes are observed with the emulsion technique, penetration at the charged oil-water interface has taken place.

Adsorptions both at the charged oil-water, and air-water interfaces are conditioned by pH and are related to the charge of the protein coming in contact with the interface. The emulsion flocculation range which has been indicated in Fig. 1-5 show that as soon as re-dispersion of the emulsion is noticed the pressure of the monolayer falls markedly, and this occurs on the side of the isoelectric point where the protein and monolayer are similarly charged.

Similar to the flocculation of emulsions, proteins can be reversibly precipitated by oppositely charged long-chain ionic compounds. Furthermore several authors have shown that soluble lipo-protein associations can exist over the pH range where the proteins and

<sup>11</sup> Frazer and Stewart, *Brit. J. Expt. Path.*, 1940, **21**, 361.

long-chain ions are similarly charged.<sup>12</sup> This observation is confirmed by the present work, where the existence of "bound protein" is demonstrated at the non-reactive pH range of the proteins.

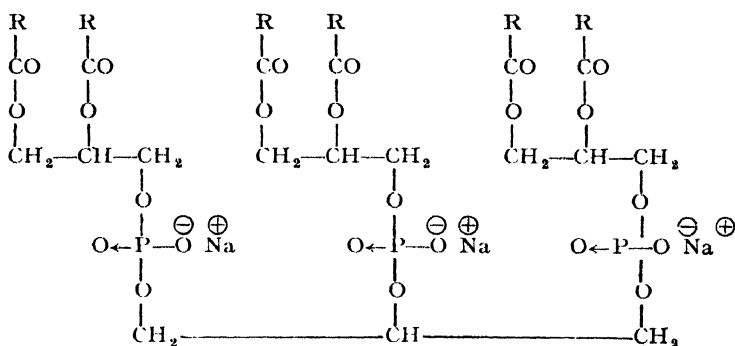
Our thanks are due to Dr. I. S. Longmuir for haemoglobin preparations, and to Mr. A. Dunn for technical assistance.

*Department of Colloid Science,  
The University,  
Cambridge.*

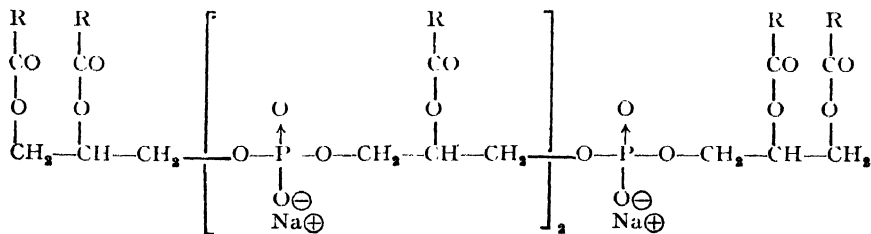
<sup>12</sup> Steinhardt and Fugitt, *J. Res. Nat. Bur. Stand.*, 1942, **29**, 315. Steinhardt, Fugitt and Harris, *ibid.*, 1941, **26**, 293.

### GENERAL DISCUSSION\*

**Dr. J. Glazer** (*Cambridge*) said: Doty, Matalon and Schulman have described and characterized the different types of affinity exhibited by



STRUCTURE A  
(after Doty and Schulman)



STRUCTURE B  
(after Pangborn)

certain proteins towards charged monolayers, one of which was cardiolipin. Doty and Schulman observed that monolayers of cardiolipin on acetate buffer (pH 5.1) collapsed at an area of 185 Å<sup>2</sup> per molecule, thereby providing strong evidence for the existence of six unsaturated long chains in the molecule, each of which is known to occupy *ca.* 30 Å<sup>2</sup> at the collapse point. They suggested that the monolayer behaviour seemed more consistent with a structure A involving three glyceryl phosphoric acid units joined to one glycerine molecule, rather than the linear structure B proposed by Pangborn.<sup>1</sup> (See structural diagrams A and B.)

\* On two preceding papers.

<sup>1</sup> Pangborn, *J. Biol. Chem.*, 1947, **168**, 351.



In choosing between these basic structures, the fact that no mono-glyceryl triphosphates have ever been isolated from natural sources or prepared synthetically makes it rather unlikely that the structure A is correct. The method of molecular models shows that while such a structure is consistent with a globular type of molecule, in which the long-chain units radiate out symmetrically from the central glyceryl triphosphate core, it would be quite impossible for a molecule having this structure to unfold so as to produce a stable monolayer. Furthermore, as will be shown below, the Pangborn structure B is fully consistent with the monolayer properties of cardiolipin, although it would seem that a modification, involving intramolecular electrostatic cross-linking between adjacent phosphate groups, is necessary to explain several of its properties.

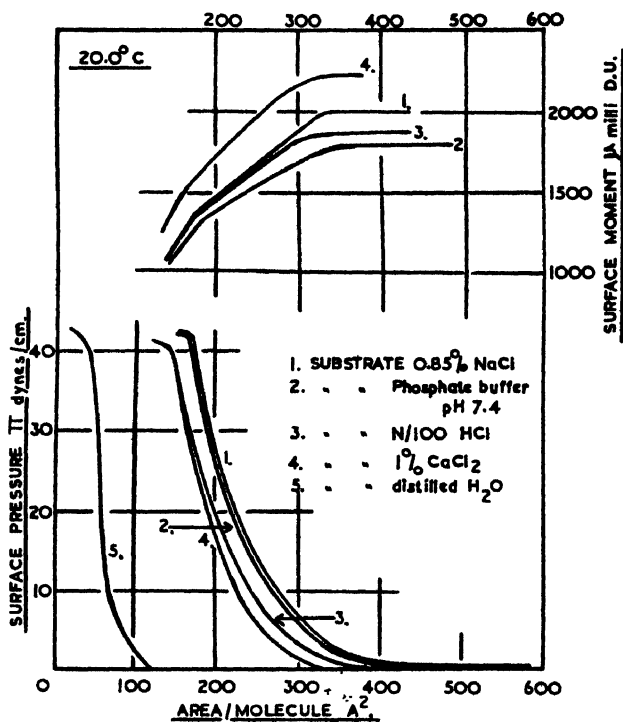
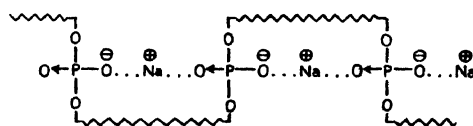


FIG. 1.

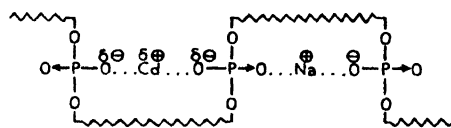
A more detailed investigation has now been made of the monolayer properties of cardiolipin, using a Langmuir-Adam trough which permitted simultaneous measurements of surface pressure and surface potential. The results are shown in Fig. 1.

The phospholipid was spread from ethyl alcohol solution (0.76 mg./ml.) on to substrates of varying pH and containing various salts. It was found that cardiolipin does not spread completely on distilled water, whereas the presence of salts in the aqueous substrate results in complete spreading. When cardiolipin is spread on distilled water and the substrate is then injected with phosphate buffer, the cardiolipin remains on the surface in its unfolded form. A subsequent spread of cardiolipin on to the same phosphate substrate results in complete spreading, the resulting monolayer having an area (at the collapse point) of  $180 \text{ Å}^2$  per molecule. Complete spreading was found to take place on M/100 HCl, M/20 phosphate buffer (pH 7.4), M/100  $\text{CaCl}_2$  (pH 8.5), M/7 NaCl (pH 8.0) and M/20 borate buffer (pH 10.1), whereas incomplete spreading took place on distilled water and M/5000 HCl substrates. It can be seen,

therefore, that the ethyl alcohol present in the spreading solution is responsible for the initial spreading process, while the presence of electrolytes in the aqueous substrate is necessary for the stabilization of the completely spread monolayer at the surface. (It should be mentioned that the instability of the cardiolipin monolayer is due to the spontaneous accumulation of three-dimensional islands or conglomerates in the surface. This type of spontaneous collapse is, in general, observed when the intermolecular attraction of the monolayer exceeds the adhesional attraction between the monolayer and the aqueous substrate.) The nature of this stabilization is a matter for discussion, but it seems reasonable to ascribe it to the disruption, by the dissolved electrolyte, of interphosphate cross-links in the cardiolipin molecule. It is suggested that cardiolipin, under normal conditions (i.e. in the folded state), contains the following type of intra-molecular cross-linking involving a "sodium ion" bridge, as in structure c, the function of the positive sodium ion being to link together a formally negatively charged oxygen ion with a semi-polar negative oxygen atom. Should these cross-links be sufficiently strong to overcome the affinity of the sodium ion and phosphate group for the aqueous substrate, then the molecule will not unfold completely to form a monolayer; this is found experimentally on distilled water and M/5000 HCl. The presence of sufficient electrolyte in the aqueous substrate serves to disrupt the cross-links by increasing the adhesion of the ionic part of the cardiolipin to the aqueous phase, as a result of ion-ion attraction.



STRUCTURE C



STRUCTURE D

Reference to Fig. 1 shows that cardiolipin forms liquid-expanded monolayers on aqueous substrates containing electrolytes. The surface characteristics are summarized in the following Table. The limiting

Substrate	Lim. Area at low Pressure (Å² per mol.)	Dipole at low Pressure (Milli-D)	Area at Collapse (Å² per mol.)	Dipole at Collapse (milli-D)
0.85 % NaCl . . . . .	350	2000	180	1400
Phosphate buffer (pH 7.4) . . . . .	350	1800	184	1300
1 % CaCl₂ . . . . .	340	2450	160	1500
M/100 HCl . . . . .	310	1850	175	1320

areas, both at zero pressure and at the collapse pressure, are best characterized by discontinuities in the surface dipole (rather than the surface pressure), since this function is appreciably linear over considerable ranges. From a consideration of both the above Table and Fig. 1, it is clear that the nature of the electrolyte exerts only minor effects on the

monolayer characteristics. Nevertheless, these effects are real and definite. With the exception of the  $\text{CaCl}_2$  substrate (see below), the monolayer collapses at an area of  $180 \pm 5 \text{ \AA}^2$  per molecule. This confirms the observation of Doty and Schulman on acetate buffer pH 5.1, and corresponds well to the area occupied by six close-packed unsaturated long chains at the air-water interface (i.e.  $30 \text{ \AA}^2$  per long chain); triolein, for example, occupies  $95 \text{ \AA}^2$  per molecule at the collapse point. Furthermore, the limiting area at low pressure of cardiolipin is, with the exception of M/100 HCl, *ca.*  $350 \text{ \AA}^2$  per molecule. The latter area is appreciably larger than that of triolein ( $43 \text{ \AA}^2$  per oleyl chain), and is attributable to the existence of the strong negative charge in the monolayer which, as a result of mutual repulsion, produces a more expanded film. It is significant that on a substrate of appreciable acidity, such as M/100 HCl, where this negative charge is somewhat discharged (cf.  $\alpha$ -glyceryl phosphoric acid,  $\text{pK}_a^1 = 1.40$ ), the monolayer is more condensed in the low-pressure region.

The presence of a divalent cation such as calcium is seen to effect marked condensation of the film, the area at the collapse point being only 160 per  $\text{ \AA}^2$  mol. This effect is characteristic of calcium substrates in contact with negatively charged monolayers, such as fatty acids. Pangborn<sup>2</sup> noticed that it was never possible completely to replace the sodium of cardiolipin by cadmium; she remarked that the cadmium salt of cardiolipin was always found to contain one atom of sodium together with one atom of cadmium. This is readily understood in terms of the above postulated cross-links, where a divalent cation such as cadmium is able to act as a bridge between two formally negatively charged oxygen ions, as in structure D. This divalent type of cross-link is to be distinguished from the monovalent sodium type in structure C, since the former is partially covalent in character, while the latter retains the whole of its electrostatic character. The tendency to assume this type of linkage at the air-water interface may very well account for the observed condensation when calcium ions are present in the substrate. This condensation, with consequent loss of electrostatic charge, is further characterized by the increased positive surface dipole, corresponding to the partial removal of a negatively charged layer in the surface.

The above experiments show, therefore, that the structure of cardiolipin, when spread completely at the air-water interface, is fully consistent with the structure B, it being understood that the polyglyceryl phosphate skeleton is lying in the interface with the unsaturated long chains pointing away from the aqueous phase.

**Prof. F. Haurowitz** (*Bloomington, Indiana, U.S.A.*) said: It is very difficult to understand the formation of ten layers of unfolded denatured haemoglobin around the lipid droplets. The parahaematin spectrum cannot be considered as a proof for unfolding of the protein molecules. Parahaematin spectra would also be observed if haem were detached temporarily from the globin surface and the binding surface of the globin molecule altered slightly so that the original globin-haem bond would not be reconstituted.

**Miss M. Pangborn** (*New York*) said: The evidence presented by Dr. Doty and Schulman and by Dr. Glazer on the structure of cardiolipin is most helpful. The linear structure of the polyglycerophosphoric ester which I suggested in 1947 represented merely the simplest way of accounting for the analytical composition and hydrolytic products found; there was no evidence at that time regarding the position of the linkages.

The report of the composition of the Cd salt which still contained Na probably should not be used as an argument for structural considerations. This finding was quoted from my first paper, when the purification methods were rather inadequate. The fatty acid in cardiolipin is linoleic rather

than oleic, and I wonder whether the properties of the monolayer are affected by the second double bond.

**Prof. J. R. Marrack** (*London*) said : It is probable that the antibody in syphilitic serum will combine with cardiolipin even when the serum is highly diluted, although no floccules are formed. The essential point is the amount of cardiolipin per cm.<sup>2</sup> of surface. The concentration of antibody in the experiments of Dr. Doty and Dr. Schulman must be a small fraction of a microgram per ml. ; this may be too little to have an appreciable effect on the layer of cardiolipin, even if it does combine. It is unlikely that effects would be detected when whole serum is used, as the inert proteins of serum exceed the antibody in a ratio of over 100 to 1.

Failure of antibodies to combine with antigens spread on a surface may be due to distortion of the spatial arrangement on which specific combination depends.

**Dr. J. Glazer** (*Cambridge*) said : In reply to Miss Pangborn, the monolayer compression curves of linoleic and oleic acids are practically identical. I am, of course, aware that the fatty acid content of cardiolipin is linoleic rather than oleic, but the absence of any monolayer information concerning glyceryl trilinoleate forces comparison with the corresponding trioleate. The monolayer similarity between linoleic and oleic acid suggests that this procedure is not unreasonable.

Regarding the composition of the cadmium salt of cardiolipin, I agree that caution should be exercised. It would be of interest to know if more recent analytical results are yet available, since it seems more than coincidental that the Na/Cd ratio should be unity.

**Prof. A. C. Frazer** (*Birmingham*) said : No association can be demonstrated between lecithin and protein using the techniques described by Dr. Schulman and Dr. Matalon. There is, however, evidence of a close association between lecithin and protein in the blood and this association markedly alters the properties and behaviour of the individual components. This association can be demonstrated by the effect of lecithinase on chylomicron stability and the separation of lipid and protein elements in the Nagler reaction. Lipids or proteins by themselves give rise to quite different characteristics in artificial emulsions. Presumably different types of association are being studied under these different conditions. Failure to obtain association of added lecithin with plasma protein might be due to the fact that lecithin is already present in natural plasma proteins. There is no reason to suppose that the destruction of this natural association by fractionating procedures should be readily reversible by the simple addition of lecithin.

**Dr. A. S. McFarlane** (*London*) said : I can confirm that lecithin does not combine with serum proteins. No-one disputes Prof. Frazer's point that lecithin is bound to proteins in natural serum, but in attempts to emulsify lecithin by various methods with normal or lipid-poor sera Mrs. Davey and I find that the lecithin always migrates in the electrophoresis cell separately from the serum proteins. This applies to egg and brain lecithins.

**Miss N. M. Czeczowicka** (*London*) said : Is there any evidence that the "residual protein bound in lipid" is not just surface-denatured protein and therefore cannot be resolubilized ?

**Dr. R. Matalon** (*Cambridge*) said : Surface denaturation of the protein consists of the spontaneous unfolding of the protein, and this cannot take place under the experimental conditions, as the spontaneous spreading of the protein is prevented by the high pressure which is established in the monolayer.

In reply to Prof. Frazer, the presence of lecithin in serum proteins could be explained by the solubility mechanism which has been described in our paper for cholesterol and haemoglobin.

**Dr. J. H. Schulman** (*Cambridge*) said : In reply to Prof. Frazer, by surface methods lecithin monolayers have been shown to be non-reactive

to serum proteins over the pH range 3-10, but can be readily shown to interact with snake venom injected into the underlying solution to form a lysolecithin monolayer.

In reply to Prof. Haurowitz, the desorbed haemoglobin from the emulsion surface not only shows the pure haematin spectrum, but flocculates at the isoelectric point of haemoglobin. Further, the desorbed monodisperse protein solution shows the colour change to reddish brown on reduction by sodium bisulphate, on warming and on pH change to alkali solution.<sup>2</sup> The restabilization of the emulsion on desorption of the protein is readily explained by the reversible adsorption of the haemoglobin from a negatively charged surface on changing the pH of the solution to a pH above the isoelectric point of the protein.

<sup>2</sup> Elkes, Frazer, Schulman and Stewart, *Proc. Roy. Soc. A*, 1945, **184**, 102.

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## THE COMBINATION OF FATTY ACID ANIONS WITH PROTEINS

BY J. MURRAY LUCK

*Received 20th May, 1949*

Anions of the aliphatic and aromatic carboxylates, sulphonates, and sulphates are bound by the serum albumins more markedly than by other proteins reported upon to date. The number of ions bound per mole of serum albumin, as well as the contribution of the ion to the thermal stability of aqueous solutions of serum albumin, is a function of the length of the side chain. Maximal effects in the case of fatty acid anions are observed with chains of six to nine carbon atoms. Protection against the denaturation of serum albumin by urea is also conferred by the family of anions mentioned. It is concluded that the binding of fatty acid anions at pH 7.5 to 8.0 is due to electrostatic attraction by the positively charged guanidine and lysine residues and van der Waals' forces between the side chains of the added anion and the side chains of leucine, isoleucine, valine, and phenylalanine.

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The present paper is devoted to recent work on the binding of ions by proteins. It is largely restricted to the binding of fatty acid ions by serum albumin and, possibly with undue attention, to studies that have proceeded in the author's laboratory. The first of these restrictions is deliberately imposed by a desire not to wander too far from the general subject of the Discussion and also by the rather curious specificity of the phenomenon itself to which we shall presently return.

But first let us address to ourselves a very pertinent question: what experimental findings necessitate the conclusion that ions are bound by proteins? Some of the evidence comes from ultrafiltration<sup>1, 2</sup> and dialysis-equilibrium studies,<sup>3, 4, 5</sup> effects on absorption spectra,<sup>6, 7</sup>

<sup>1</sup> Boyer, Ballou and Luck, *J. Biol. Chem.*, 1947, **167**, 407.

<sup>2</sup> Greenberg and Gunther, *ibid.*, 1929-30, **85**, 491.

<sup>3</sup> Klotz, Walker and Pivan, *J. Amer. Chem. Soc.*, 1946, **68**, 1486.

<sup>4</sup> Teresi and Luck, *J. Biol. Chem.*, 1948, **174**, 653; 1949, **177**, 383.

<sup>5</sup> Klotz, Triwush and Walker, *J. Amer. Chem. Soc.*, 1948, **70**, 2935.

<sup>6</sup> Klotz, *ibid.*, 1946, **68**, 2299.

<sup>7</sup> Irvine and Irvine, *Fed. Proc.*, 1949, **8**, 209.

and binding studies on dyes,<sup>8, 9, 10</sup> on indicators,<sup>11-14</sup> and on synthetic detergents.<sup>15-19</sup> In addition, Kendall<sup>20</sup> has described a serum albumin which contained 2 % fatty acid—somewhat more than the crystallized albumins prepared by the ethanol procedure. Macheboeuf's studies<sup>21</sup> on protein-fatty acid complexes are well known and add to the body of direct evidence.

Equally convincing evidence consists in part of an observation by Scatchard and Black<sup>22</sup> that serum albumin solutions, rendered iso-ionic by exhaustive dialysis against water, undergo an increase in pH of as much as 1.65 pH units by addition of various neutral inorganic salts. This is suggestive of anion binding and of a considerable change in the ionic properties of the molecule. Studies recently reported by Longworth and Jacobsen<sup>23</sup> and by Velick<sup>24</sup> likewise give evidence of protein-anion combinations, sometimes of a clearly competitive character. The electrophoretic mobility of serum albumin, determined after equilibration with sodium salts of the lower fatty acids, increases with increase in chain length of the added salt.<sup>25</sup> This is strongly suggestive of an anion-albumin association. The results, however, do not permit of an indubitable conclusion since phosphate which also increases the net negative charge of serum albumin is found by Teresi,<sup>26</sup> using another method, to bind with serum albumin to a degree less than would be predicted from the electrophoretic findings. Some years ago we observed by means of a so-called cloud-point technique that the thermal stability of serum albumin in aqueous solution was much increased by fatty acid anions,<sup>1, 27, 28, 29</sup> the effect increasing with increase of chain length. I doubt that I would be inclined to cite this as conclusive evidence of fatty acid binding were it not that this method of study shows the same effects of chain length and side-chain polar groups as ultra-filtration,<sup>1</sup> and stabilization of serum albumin against urea denaturation as studied by viscosimetry.<sup>30, 31</sup> Further indirect evidence that points to the same conclusion is found in many current studies akin to those of Davis and Dubos<sup>32</sup> in which it was observed that the salutary effects of serum albumin on the growth of

<sup>8</sup> Chapman, Greenberg and Schmidt, *J. Biol. Chem.*, 1927, **72**, 707.

<sup>9</sup> Rawlins and Schmidt, *ibid.*, 1929, **82**, 709; 1930, **88**, 271.

<sup>10</sup> Stern, *J. Physic. Chem.*, 1930, **34**, 973, 980.

<sup>11</sup> de Haan, *J. Physiol.*, 1922, **56**, 444.

<sup>12</sup> Grollman, *J. Biol. Chem.*, 1925, **64**, 141.

<sup>13</sup> Marshall and Vickers, *Bull. Johns Hopkins Hosp.*, 1938, **34**, 1.

<sup>14</sup> Smith and Smith, *J. Biol. Chem.*, 1938, **124**, 107.

<sup>15</sup> Lundgren, Elam and O'Connell, *ibid.*, 1943, **149**, 183.

<sup>16</sup> Lundgren and O'Connell, *Ind. Eng. Chem.*, 1944, **36**, 370.

<sup>17</sup> Putnam and Neurath, *J. Amer. Chem. Soc.*, 1944, **66**, 692, 1992.

<sup>18</sup> Neurath and Putnam, *J. Biol. Chem.*, 1945, **160**, 397.

<sup>19</sup> Lundgren, *J. Textile Res.*, 1945, **15**, 335.

<sup>20</sup> Kendall, *J. Biol. Chem.*, 1941, **138**, 97.

<sup>21</sup> Macheboeuf and Tayeau, *Bull. Soc. Chem. Biol.*, 1941, **23**, 49.

<sup>22</sup> E.g. Scatchard and Black, *J. Physic. Chem.*, 1949, **53**, 88.

<sup>23</sup> Longworth and Jacobsen, *ibid.*, 1949, **53**, 126.

<sup>24</sup> Velick, *ibid.*, 1949, **53**, 135.

<sup>25</sup> Ballou, Boyer and Luck, *J. Biol. Chem.*, 1945, **159**, 111.

<sup>26</sup> Teresi (unpublished observation).

<sup>27</sup> Ballou, Boyer, Luck and Lum, *J. Clin. Invest.*, 1944, **23**, 454.

<sup>28</sup> Ballou, Boyer, Luck and Lum, *J. Biol. Chem.*, 1944, **153**, 589.

<sup>29</sup> Boyer, Lum, Ballou, Luck and Rice, *ibid.*, 1946, **162**, 181.

<sup>30</sup> Boyer, Ballou and Luck, *ibid.*, 1946, **162**, 199.

<sup>31</sup> Duggan and Luck, *ibid.*, 1948, **172**, 205.

<sup>32</sup> Davis and Dubos, *J. Expt. Med.*, 1947, **86**, 215.

the tubercle bacillus *in vitro* were due to the albumin fixation of oleic acid (and perhaps other unsaturated acids), present as a contaminant in the nutrient medium. Related, no doubt, is the protective effect of serum albumin against haemolysis *in vitro* by various fatty acids. Our own studies<sup>1</sup> of this phenomenon, restricted to sodium caprylate, were pursued with the hope that the quantitative findings would agree with other methods and would permit the application of this simple and rapid technique to other proteins, other fatty acids, and other ions. However, the protective action of caprylate was greater than that which would have been predicted from the "combined caprylate" content of the medium, independently determined by ultra-filtration.

Many observations<sup>23, 24, 28-38</sup> have been made in electrophoretic studies of effects, sometimes specific, of buffer anions on the mobility and iso-electric or iso-ionic point of a protein. Although in many of the cases reported a protein-anion interaction is in evidence the observations are not readily interpreted; the size and valency of the anion, the ionic strength and pH of the solution, the nature of the protein component, competition between the various ionic species present, and simultaneous proton binding by the COO<sup>-</sup> groups of the protein are recognized as important variables.

Of the methods mentioned for studying fatty acid binding, the ultra-filtration and dialysis-equilibrium procedures are among the most satisfactory. Boyer,<sup>1</sup> formerly in our laboratory, has used the first of these to considerable advantage in studying the binding of butyrate, caproate, caprylate, caprate, and acetyltryptophan. Noda<sup>39</sup> extended its use to mandelate. Results obtained by this method are amenable to quantitative interpretation since a simple mass action expression is found to be applicable. Higher concentrations of protein and ion may be employed than in the usual type of dialysis-equilibrium study: this, in turn, sometimes permits the use of analytical methods which are insufficiently sensitive for application to the low concentrations used in dialysis-equilibrium investigations. Specifically, we are not yet able to carry out binding studies with unlabelled aliphatic anions by the latter method owing to their lack of absorption in the visible or ultraviolet, but we are able to do so by simple acid-base titration with the higher protein concentrations characteristic of ultra-filtration studies. I suppose it may also be argued that the protein concentrations used in the latter more nearly approach serum protein values and that results obtained may therefore be more significant in connection with the transport function now commonly assigned to serum albumin.<sup>40</sup>

The dialysis-equilibrium method was introduced to this field of investigation quite some years ago. v. Muralt<sup>41</sup> developed a mathematical treatment based upon the law of mass action, applicable to the binding of hydrogen ions and clearly capable of extension to the multiple binding of other ions where a series of association constants is involved. This extension was effected and the treatment somewhat

<sup>23</sup> Moyer, *Trans. Faraday Soc.*, 1940, **36**, 248.

<sup>24</sup> Moyer and Moyer, *J. Biol. Chem.*, 1940, **132**, 373.

<sup>25</sup> Sookne and Harris, *J. Res. Nat. Bur. Stand.*, 1939, **23**, 299.

<sup>26</sup> Davis and Cohn, *J. Amer. Chem. Soc.*, 1939, **61**, 2092.

<sup>27</sup> Longworth, *Ann. N.Y. Acad. Sci.*, 1941, **41**, 267.

<sup>28</sup> Alberty, *J. Physic. Chem.*, 1949, **53**, 114.

<sup>29</sup> Noda, unpublished observations, see Luck, *ibid.*, 1947, **51**, 229.

<sup>40</sup> Davis, *Amer. Scientist*, 1946, **34**, 611.

<sup>41</sup> v. Muralt, *J. Amer. Chem. Soc.*, 1930, **52**, 3518.

simplified by Klotz<sup>3</sup> and used in dialysis-equilibrium studies on proteins by various investigators. The results obtained lend themselves readily to conventional thermodynamic treatment despite the present inadequacy of all attempts to describe adequately the binding centres and to define precisely the character of the bonds that are formed. The equations developed by Klotz<sup>42</sup> permit an evaluation of the role of statistical and electrostatic factors in binding, and the determination in most cases of the number of ions bound per mole of protein and the bond energies. Some of Klotz's most interesting findings are derived from the binding of methyl orange and azosulphathiazole, but it seems probable that some of his conclusions are applicable also to fatty acid binding. However, it appears that van der Waals' forces play a much more important role with fatty acids than with azo compounds—a point to which we shall presently return.

It may now be of interest to inquire whether the ions bound by the serum albumins, at least, have any characteristic and distinguishing qualities. If we restrict the problem to systems in solution and exclude ion-albumin complexes of very low solubility in water, such as some of the metallic salts, one or two generalizations appear to be inescapable. First of all it is increasingly apparent that the serum albumins have a singularly conspicuous capacity to bind non-polar anions. The aliphatic and aromatic carboxylates, sulphonates, and sulphates are strongly bound if the side chain is sufficiently long and is virtually free of polar groups. The introduction of hydroxy groups or amino groups reduces the binding. Mandelate is bound slightly as compared with its homologue, phenylacetate.  $\alpha$ -Amino acids are bound to a negligible extent, if at all, but the acetylated amino acids such as acetyltryptophan are quite appreciably bound and may, for example, displace methyl orange.<sup>5</sup> Binding is also in evidence in the case of a number of organic ions which lack the marked non-polar properties of the other ions mentioned: e.g. 2:4-dichlorophenolate, 2:4-dinitrophenolate, the three mononitrophenolates, picrate, and trichloroacetate. The binding of organic anions by serum albumin takes place over a wide pH range, although most of our studies have been carried out at pH 7.5 to 8.2. In this region the positive charges are localized in the guanidine and lysine side chains of the protein molecule. The binding of inorganic ions, to which increasing attention is now being given,<sup>22</sup> is deliberately omitted from this paper.

If it be assumed for the moment that the binding of organic anions is partly electrostatic it would seem reasonable to expect that organic cations, of side chain structure similar to the organic anions, would also be bound: the side-chain free carboxyl groups of aspartic and glutamic acids are fully ionized at pH 7.8 and the number of such groups is about as great as the number of basic groups—130 to 135 per mole of serum albumin; the low dipole moment of the protein suggests, furthermore, that the positively and negatively charged groups are fairly evenly distributed over the surface of the molecule. We have completed, however, an extensive study<sup>43</sup> of many aliphatic monoamines, from  $C_4$  to  $C_{12}$ , and of several di-amines without observing any comparable phenomenon: viscosity studies failed to reveal any appreciable stabilization against urea denaturation, and cloud-point studies revealed a heightened susceptibility to heat denaturation. We

<sup>42</sup> Klotz, *Arch. Biochem.*, 1946, 9, 109.

<sup>43</sup> Luck and Welsh (unpublished observations).



have not employed with amines the quantitative dialysis-equilibrium techniques and shall have to postpone such studies until we have several radioactive amines or suitable microanalytical methods for the unlabelled substances.

The next question I would like to consider is whether the ions bound by serum albumin, especially the organic non-polar anions, are bound by other proteins to a comparable degree. Our own findings indicate that bovine serum albumin binds about 25 ions per mole in the case of the more strongly associated anions,<sup>4</sup> crystalline  $\beta$ -lactoglobulin<sup>28</sup> about 2, and crystalline  $\beta$ -amylase<sup>28</sup> none. Klotz<sup>44</sup> divides the proteins he has studied into three groups on the basis of their relative binding capacities: serum albumin and  $\beta$ -lactoglobulin in the first, ovalbumin and conalbumin in the second, and pepsin, trypsin, chymotrypsin, ribonuclease, and insulin in the third (no binding). Although serum albumin and  $\beta$ -lactoglobulin are grouped together Klotz recognizes that the latter is much inferior to serum albumin in binding capacity. Davis and Dubos report<sup>32</sup> that the protective action of serum albumin against oleic acid, as observed in cultivation of the tubercle bacillus, was evidenced by  $\beta$ -lactoglobulin to a slight degree and was not displayed by other proteins that were tried. A study of the spectral shifts due to complex formation between proteins and azo dyes has been carried out by Klotz.<sup>6</sup> By displacement analysis the competitive effects of a number of simple anions have been investigated. Germane to our present point is the observation that spectral shifts were not observed when serum albumin was replaced by gelatin or  $\gamma$ -globulin: evidently binding did not occur. Our own cloud-point studies cause us to exclude serum  $\gamma$ -globulin, insulin, diphtheria toxin, diphtheria antitoxin, and papain though we recognize that the evidence by this method is suggestive and not conclusive. It appears, then, that we are concerned with a phenomenon which is peculiar to the serum albumins.

In seeking an explanation for this specificity it is necessary next to inquire into the mechanism of binding. For example, it would be pertinent to ask ourselves whether electrostatic forces, expressed by a straightforward salt linkage between the anions and positively charged groups on the protein, play an essential role. Part of the answer is to be found in the behaviour of certain amides and esters. Here again we have only the indirect evidence contributed by thermal stability studies. Ethyl butyrate, butyramide, caproamide, monocaproin, monocaprylin, monocaprin, and triacetin all have quite small stabilizing effects with solutions of crystalline human serum albumin; none has an effect as great as even butyrate and most of them are about as effective as chloride.<sup>29</sup> The results with the monoglycerides are not unequivocal because of the polarity of the glycerol residue: we would now expect such compounds to be less effective for this reason alone. With caproamide, however, no such property is in evidence and a simple comparison with caproate obliges us to conclude that the substance must be ionic if it is to be bound.\* Inferentially, we next conclude that binding with positively charged groups is essentially what happens.

<sup>44</sup> Klotz, *San Francisco Meeting, Amer. Chem. Soc.*, March 29, 1949.

\* This may not be rigorously true since very small amounts of aliphatic alcohols ( $C_6$  to  $C_{10}$ ), benzene, toluene, chloroform, and ethylene dichloride when used to facilitate the crystallization of serum albumin have been found by

The direct evidence of participation of basic groups is found in studies on acetylated serum albumin and formaldehyde-treated protein. Teresi<sup>46</sup> finds that elimination of the free  $\epsilon$ -amino groups of lysine by this treatment reduces the number of ions bound per mole of protein in the case of *m*-nitrophenolate and *p*-nitrophenolate. The three *o*-nitrophenolates (mono-, di-, and tri-nitro) suffered no reduction in binding by treatment of the protein with formaldehyde or acetylation. This suggests that the binding of these last-mentioned substances may be a function of other groups, e.g., guanidine residues. To test the preceding hypothesis, Teresi is now investigating the behaviour of guanidinated serum albumin which, if the hypotheses be true, should bind increased quantities of the ions mentioned. Klotz has also found<sup>44</sup> that the acetylation of serum albumin greatly reduces its capacity to bind methyl orange.

The role of van der Waals' forces into which we might next inquire is important. That they play a conspicuous part is clearly evident from the work of Boyer<sup>1</sup> on the effect of chain length on the binding of aliphatic carboxylates and from the indirect evidence afforded by cloud-point studies: increase of chain length in the homologous series (up to C<sub>10</sub>) of aliphatic carboxylates increases the capacity of these substances to stabilize solutions of serum albumin against thermal or urea denaturation. The role of van der Waals' forces is lessened as we turn to the azo dyes or to ions with side-chain polar groups. With the alkyl sulphates and serum albumin the role of van der Waals' forces is not clearly established, as witness the confusing results of Karush and Sonenberg<sup>48</sup> with octyl, decyl and dodecyl sulphates.

It seems improbable that quantitative evaluations<sup>3</sup> of the relative contributions of electrostatic forces and van der Waals' forces are of general significance since the values are determined by the nature of the ion under consideration. The contribution of hydrogen bonding as between side-chain groups and carboxyl, hydroxy, and imino groups in the albumin molecule has not been determined. Indeed, to consider the nature of the binding forces satisfactorily and to determine, for stated anions, the contribution of each (electrostatic, van der Waals', and hydrogen bonds) we would have to know much about the topography of the protein surface that is still in the unknown: the distribution of the quaternary nitrogens, and their distance from such influential neighbours as the free carboxyls of glutamic and aspartic acid, the hydroxyl groups of serine, threonine, and tyrosine, and the non-polar side chains of such amino acids as leucine, isoleucine, valine, and phenylalanine.

The binding energies for various ions in combination with serum albumin are expressed in Table I. The maximum possible number of ions bound per mole of protein are also presented as  $n$  values<sup>3</sup> where  $n$  is given by the equation  $k_1 = n/K$ .  $\Delta F_1$  is calculated from the first equilibrium constant ( $k_1$ ) in the series of reactions  $P + A \rightleftharpoons PA$ ;  $PA + A \rightleftharpoons PA_2$ ; . . . ;  $PA_{i-1} + A \rightleftharpoons PA_i$ ; . . . ;  $PA_{n-1} + A \rightleftharpoons PA_n$ .

Cohn *et al.*<sup>45</sup> to be associated with the crystalline product. Whether or not these substances are combined with the albumin in solution is undetermined. Bilirubin is also bound, though it appears that the binding capacity of serum  $\alpha$ -globulin is appreciably greater than albumin.<sup>47</sup>

<sup>45</sup> Cohn, Hughes and Weare, *J. Amer. Chem. Soc.*, 1947, **69**, 1753.

<sup>46</sup> Teresi, *Portland Meeting, Amer. Chem. Soc.*, September, 1948.

<sup>47</sup> Nicholas, *J. Amer. Chem. Soc.*, 1949, **71**, 1230.

<sup>48</sup> Karush and Sonenberg, *ibid.*, 1949, **71**, 1369.

From the nitrophenolate series it would appear that increase in ionization increases, as might be expected, the binding energy; this is further increased by the introduction of a second or third nitro group. The behaviour of *o*-nitrophenolate is unlike that of the *m*- and *p*-isomers both in respect to  $n$  and  $-\Delta F_1$ .

At this point it may be worth asking ourselves whether, in the serum albumin-anion complex, the protein is present in the native form or the open extended form characteristic of the denatured molecule. Much seems to depend upon the nature of the anion. For example with caprylate, as used in many of our studies, there seems to be no doubt that the association complex is built up from native serum albumin in the closed configuration. So also with dodecyl sulphate in low concentrations. This is proven by viscosity studies, even in the presence of high concentrations of urea and, for caprylate also, by rate of digestion with papain. With certain other ions, however,

TABLE I.—BINDING ENERGIES WITH SERUM ALBUMIN

Anion	A <sup>-</sup> Form per cent.	pH	Bond Energies ( $-\Delta F_1$ ) Calories per mole	$n$	Reference
Chloride . . .	—	—	1,800	—	43
Sulphanilamide . . .	—	—	3,740	—	43
Salicylate . . .	—	—	4,780	—	43
<i>o</i> -Nitrophenolate . . .	75	7.5	5,565	—	4
	91	8.2	5,455	—	4
<i>m</i> -Nitrophenolate . . .	16	7.5	4,965	24	4
	43	8.2	5,615	22	4
<i>p</i> -Nitrophenolate . . .	73	7.5	4,865	25	4
	91	8.2	5,465	25	4
2 : 4-Dinitrophenolate . . .	100	—	6,490	—	4
Picrate . . .	100	—	6,590	—	4
Phenyl acetate . . .	—	—	4,015	25	4
Phenoxyacetate . . .	—	—	4,865	7	4
Cinnamate . . .	—	—	5,465	25	4
Methyl orange . . .	100	—	5,960	25	43
Dodecyl sulphate . . .	100	—	~10,000	—	43

notably mandelate, no protection is conferred against urea denaturation: the relative viscosity of the solution is no less than in the absence of mandelate. The same seems to be true of benzoate, benzene sulphonate, phenylacetate, and acetyltryptophan. In these cases we are drawn to the conclusion that while, under ordinary circumstances, the substances mentioned may combine with albumin in its native state, under conditions that favour protein denaturation, partial opening out of the molecule proceeds: combination with the anion next takes place to give an anion-protein complex of greater solubility than the denatured protein alone. True it is that these observations were made upon albumin solutions of about 2%: it is not proven that the same conclusions would be applicable to the 0.2% solutions used in Teresi's quantitative dialysis-equilibrium studies.

In conclusion I think it desirable to speculate upon the specificity of serum albumin in so far as the binding of anions is concerned. Serum albumin is a molecule of low dipole moment with over 130 groups (guanidine and lysine) that carry a positive charge at pH 7.5 to 8.0, and an almost equal number of negatively charged groups arising

from the ionization of the free carboxyl groups of aspartic and glutamic acid residues. The low dipole moment of the protein suggests that these ionized groups are uniformly distributed over the surface. From the reported dimensions of the serum albumin molecule,  $38 \text{ \AA} \times 150 \text{ \AA}$ , assuming it to be a prolate ellipsoid, Teresi has calculated the average distances that separate, centre to centre, positive and negative charges. Whether the charges are hexagonally distributed over the surface or at the corners of squares is of comparatively little consequence. The value works out to about  $7.1 \text{ \AA}$  in either event and would be appreciably greater if half of the charges were buried beneath the surface, as may indeed be the case. In so far as this distance is maintained between neighbouring oppositely charged groups the probability of interaction is slight.

It seems not improbable, however, by virtue of the number of ionized groups involved that some, if not many of these, would be contiguous to the non-polar side-chains of leucine, *isoleucine*, valine, and phenylalanine. Since van der Waals' forces play an important role in the binding of fatty acids we propose the hypothesis that the binding capacity of serum albumin is a dual function of the large number of positively charged groups and the close juxtaposition of some of these to non-polar side chains of certain amino acids, especially leucine, *isoleucine*, valine, and phenylalanine. In using this hypothesis as a basis for predicting what other proteins would be of high binding capacity we consider that many positively charged groups would be essential; so also would it appear important that the proteins be of low dipole moment, otherwise some of the groups might be unavailable because of an inappropriate distribution over the surface of the molecule. Conceivably, proteins with an appreciable excess of positively charged groups might be of high anion-binding capacity provided that the groups concerned were well distributed over the surface of the molecule and the number of non-polar amino acid residues were not too low (as in the protamines).

Klotz<sup>43</sup> has recently proposed the theory that the binding capacity of a protein is a direct function of the number of positively charged groups and an inverse function of the number of carboxyl and hydroxy groups. Since hydrogen bonding between the carboxyl and hydroxy groups is involved the theory tacitly infers that the aspartic, glutamic, tyrosine, serine, and threonine residues are so oriented as to permit this hydrogen bonding to take place. In the absence of any evidence that such is the case, at least on the scale implicit in the theory, we are unable to regard it as an acceptable explanation of the facts. The theory also minimizes the role of van der Waals' forces. On the basis of binding studies with methyl orange at two different temperatures, Klotz has concluded on thermodynamic grounds that van der Waals' forces are of minor significance. In our experience methyl orange is atypical and conclusions drawn therefrom are not applicable to the binding of fatty acids, in so far as determination of the role of van der Waals' forces is concerned.

The author is most grateful to Dr. J. D. Teresi for permission to report upon his current investigations and for his generous assistance in preparation of this manuscript. Prof. I. M. Klotz generously made available an advance copy of his latest paper on the binding of organic ions by proteins. We are also indebted to Dr. A. K. Balls for a sample

of crystalline  $\beta$ -amylase, to Dr. W. G. Gordon for a sample of crystalline  $\beta$ -lactoglobulin, and to the Armour Laboratories for the crystalline bovine serum albumin used in our studies. The Rockefeller Foundation through a grant-in-aid has made possible these investigations.

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## THE ADSORPTION OF PARAFFIN-CHAIN SALTS TO PROTEINS

### PART V. THE INFLUENCE OF SIZE OF ION ON THE BINDING OF AMPHIPATHIC ANIONS AND CATIONS TO GELATIN

BY KENNETH G. A. PANKHURST

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A study has been made of the primary adsorption of a variety of amphipathic ions to gelatin, and it has been shown that ionic attraction between the charged side chains of the protein and the amphipathic ions, irrespective of the size of the ion, depends on the net charge on the protein and the charge on the amphipathic ions. Adsorption of amphipathic ions can also occur at some or all of the keto-imide groups of the main chain of the protein molecule by an ion-dipole association. The presence of inorganic electrolyte encourages such adsorption whereas hydrogen ions prevent it. Only when the heads of the amphipathic ions are sufficiently small (*ca.* 20-25 Å<sup>2</sup> cross-sectional area) are they able to penetrate to all the keto-imide groups. Ions with heads larger than *ca.* 45 Å<sup>2</sup> in cross-sectional area are too large to be adsorbed at any of the backbone sites. Ions of intermediate size are able to penetrate only to a limited number of these sites.

Previous work has shown that adsorption of dodecyl sodium sulphate (DSS) to gelatin takes place in two consecutive stages.<sup>1</sup> First, DSS anions are adsorbed with their polar groups towards the gelatin until a primary monolayer is built up and secondly, when this is complete, a further layer is formed with the polar groups of the detergent anions orientated outwards. Thus, on the addition of DSS to a gelatin sol, adsorption complexes are formed which become increasingly hydrophobic as the detergent/protein ratio increases and then, having reached a maximum, become more hydrophilic as the secondary layer is formed. In certain circumstances, e.g. at low pH values or in the isoelectric zone in the presence of inorganic salt, the adsorption complexes become sufficiently hydrophobic to be thrown out of solution as oil-soluble coacervates. The number of DSS anions per unit of gelatin required for the formation of a complete primary monolayer has been shown to be dependent on pH and the presence of inorganic electrolyte, and adsorption at two distinct types of site in the protein molecule have been suggested to explain the results.<sup>2</sup> The cationic side chains alone

<sup>1</sup> Pankhurst and Smith, *Trans. Faraday Soc.*, 1944, **40**, 565. Joly, *Bull. Soc. Chim. biol.*, 1948, **30**, 398.

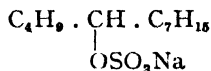
<sup>2</sup> Pankhurst and Smith, *Trans. Faraday Soc.*, 1945, **41**, 630.

are responsible for the coulombic binding of DSS anions at low pH values in the absence of inorganic salt, and a stoichiometric equivalence has been demonstrated between the number of these groups and the number of DSS anions bound in the primary layer. This number decreases as the protein is deaminated.<sup>3</sup> Indeed, the completion of the primary layer can be used to determine the number of cationic side chains in the protein molecule<sup>4</sup> in the same way as dye anions have been used.<sup>5</sup> Increasing the pH and the addition of inorganic electrolyte permits the fixation of many more DSS anions in the primary layer, indicating that fresh sites in the protein molecule become available for the primary adsorption of DSS anions. At pH values at and above the isoelectric point, in the presence of a high concentration of inorganic salt (e.g. M NaCl), primary adsorption of DSS anions is maximal, the number bound being practically equivalent to the total number of amino acid residues in the protein. The most likely sites for such adsorption to occur are the keto-imide groups in the protein backbone, adsorption being by an ion-dipole mechanism. If this is so, the degree to which these groups are capable of taking part in such a process is dependent on pH and inorganic salt, hydrogen ions reducing and inorganic salt increasing their polarity. In the present work, a study has been made of the primary adsorption of a number of different anionic and cationic paraffin-chain salts to test this hypothesis.

### Experimental

**Materials.**—The primary alkyl sulphates were prepared from carefully fractionated alcohols (b.p. range of less than 5° C) by treatment with concentrated sulphuric acid below 40° C added over a period of an hour. The reaction mixtures were neutralized, dried, extracted with dry methyl alcohol and the alkyl sulphates crystallized not less than three times from methyl alcohol. Sodium contents were all within 1 % of theory.

A secondary isomer of DSS (sodium dodecyl 5-sulphate) was prepared by sulphating the corresponding alcohol, prepared from re-distilled butyl bromide and *n*-caprylaldehyde via the Grignard reaction, with chlorosulphonic acid. This was crystallized three times from methyl alcohol. The sodium content was found to be 8.7 % whereas



gives 7.98 %. Titration against standard cetyl trimethylammonium bromide<sup>6</sup> gave a molecular weight of 268, for which Na = 8.6 %.

The alkyl naphthalene sulphonate was commercial Permal W (ex. I.C.I.) which contained 64 % of inorganic salt. When used, additions of NaCl were made to give a constant inorganic salt concentration.

Flavianic acid (2 : 4-dinitro 1-naphthol 7-sulphonic acid) (ex. B.D.H.) was used without further purification and gave a titration with NaOH equivalent to 99.3 % purity.

Cetyl pyridinium bromide and cetyl trimethylammonium bromide were prepared from fractionated cetyl bromide (see Adam and Pankhurst<sup>7</sup>).

<sup>3</sup> Harris, Pankhurst and Smith, *Trans. Faraday Soc.*, 1947, **43**, 506.

<sup>4</sup> Pankhurst, in *Surface Chemistry* (Butterworth, London, 1949) p. 109.

<sup>5</sup> Fraenkel-Conrat and Cooper, *J. Biol. Chem.*, 1944, **154**, 238.

<sup>6</sup> Epton, *Trans. Faraday Soc.*, 1948, **44**, 226.

<sup>7</sup> Adam and Pankhurst, *ibid.*, 1946, **42**, 523.

Cetylamine hydrochloride was prepared from purified ethyl palmitate via the acid, acid chloride, amide and nitrile as described by Adam and Dyer.<sup>8</sup>

Gelatin was Coignet Gold Leaf and all quantities refer to the moisture-free protein.

Measurements of the size of the detergent ions were made from Fisher-Hirschfelder atom models kindly lent by the Wellcome Foundation.

**Insoluble Complexes.**—For each pH value and inorganic salt concentration, a series of mixtures was prepared, each being identical as regards pH, added salt and gelatin concentration (0.5 %), but with varying concentrations of detergent so as completely to cover the range of water insolubility. These were left overnight at 35° C to allow the insoluble complexes to separate. The supernatant liquors were then analyzed for nitrogen (micro-Kjeldahl) and the complex of minimum water solubility, i.e. that at which the supernatant nitrogen was minimal, determined. It was found unnecessary to analyze for detergent ion in the supernatant liquor as it had been found that, except for relatively high concentrations of detergent (above *ca.* 0.1 M) all of the detergent reacts with all of the protein, the supernatant liquor being a saturated solution of the separated complex. This method can be used even if the detergent contains nitrogen since, when the primary layer is complete, both protein and detergent in the supernatant liquor are minimal and the required detergent/protein ratio is that of the two initial reactant concentrations.<sup>4</sup>

**Soluble Complexes.**—Viscosity measurements (Ostwald viscometer) of a series of mixtures of detergent and constant protein in solution, show a pronounced fall to a minimum as the detergent/protein ratio increases and then a rise.<sup>9</sup> The ratio corresponding to minimum viscosity was taken as that at which the primary layer was complete, the solute here being least lyophilic (cf. the effect of the addition of a poor solvent to a lyophilic sol<sup>10</sup>).

## Results

The main experimental results are shown in Fig. 1, in which the detergent/protein ratio (mmole/g.) corresponding to the complete primary monolayer is plotted vertically against pH for a variety of anionic and cationic detergents.

**Anionic Detergents.**—At pH 2, in the absence of inorganic electrolyte, the binding of alkyl sodium sulphates is equivalent to the total cationic side chains of the protein (0.87 mmole/g.<sup>11</sup>). The addition of inorganic electrolyte increases the number of anions bound at this pH (e.g. M NH<sub>4</sub>NO<sub>3</sub> increases the fixation of DSS fourfold).

At pH 5.5 in the presence of M NaCl, primary alkyl sulphates are bound to the extent of 10 ( $\pm$  0.5) mmole/g., the alkyl naphthalene sulphonate—6.2 mmole/g., the secondary alkyl sulphate—4.6 mmole/g., and the flavianate not at all. The binding of the primary alkyl sulphates in the presence of M NaCl is constant between pH 5.5 and 10.

**Cationic Detergents.**—At pH 12.5, cetyl pyridinium bromide and cetyl trimethylammonium bromide are adsorbed to the extent of 1.7 and 1.3 mmole/g. respectively and as the pH is reduced, adsorption decreases until at about pH 4 it reaches zero. A few experiments with cetylamine, however, showed that at pH 5.5 and 11, between 8 and 10 mmole/g. were adsorbed. A more accurate estimate of the ratio was not possible with this compound owing to its low solubility. At pH 2, cetylamine hydrochloride forms no complexes in the absence of inorganic salt.

<sup>8</sup> Adam and Dyer, *J. Chem. Soc.*, 1925, 72.

<sup>9</sup> Pankhurst, *Bull. Soc. Chim. biol.* (in press).

<sup>10</sup> Alfrey, Bartovics and Mark, *J. Amer. Chem. Soc.*, 1942, 64, 1557.

<sup>11</sup> Bowes and Kenten, *Biochem. J.*, 1948, 43, 358.

## Discussion

It has been suggested that two types of link are involved in the formation of protein-detergent complexes, (a) ion-ion, (b) ion-dipole. It would be expected that the former type would operate according to the net charge on the protein, i.e. maximum binding of detergent anions would occur at low pH values where the protein has its maximum positive charge, and maximum binding of detergent cations would occur at high pH values, the protein having its maximum negative charge. The experimental evidence with all the anions shows that at about pH 2 the number bound is equivalent to the sum of the ionized lysine, hydroxylysine, arginine and histidine side chains. Recent work by R. C. M. Smith with sulphonates of naphthalene also confirms

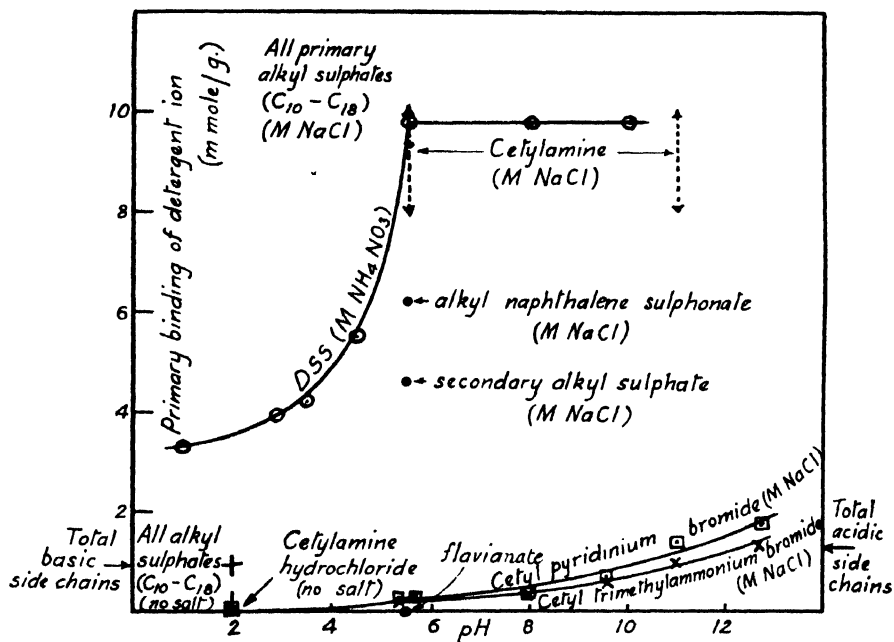


FIG. 1.

this.<sup>12</sup> Studying complexes formed with the 2-monosulphonate, 1:3-disulphonate and 1:3:5-trisulphonate, he found that, at low pH values, whereas the monosulphonate bound is equivalent to the cationic sites on the protein side chains, the di- and tri-sulphonates combine in decreasing amounts, expressed in mmole/g., in the ratio 3/2/1.

Were ionic forces solely responsible for the adsorption of long chain anions, one would expect that, as the pH is raised, the number bound would decrease. Although no detailed study of this has been made, in the absence of inorganic electrolyte, it has been shown<sup>9</sup> that at pH 5.5, in the absence of salt, soluble complexes are formed containing about 0.8 mmole/g., and that if inorganic salt is added this ratio increases to *ca.* 10 mmole/g. (observed when NaCl is 0.75 M and over). It is thus concluded that even though raising the pH may cause a reduction in the number of anions bound purely ionically, this effect is masked since other sites to which detergent anions are capable of being

<sup>12</sup> Smith, *Nature*, 1949, 164, 447.



adsorbed come into operation. Furthermore, the presence of inorganic salt tends to enhance the availability of such other sites, even at low pH values. The maximum number of bound detergent anions which has been observed is  $10 (\pm 0.5)$  mmole/g. for the primary alkyl sulphate anions at pH 5.5 in the presence of *ca.* M NaCl, which is very close to the number of amino acid residues in the protein (10.7), and suggests that the backbone keto-imide groups can act as sites for the adsorption of detergent anions, presumably by an ion-dipole mechanism.

The results with the secondary isomer of DSS, the alkyl naphthalene sulphonate and flavianic acid are interesting in that, under conditions where the primary sulphates are maximally adsorbed (pH 5.5 in the presence of M NaCl), they are bound in much smaller amounts, the latter forming no complex at all, soluble or insoluble.



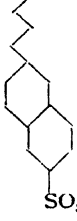

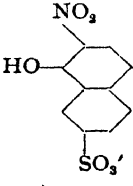
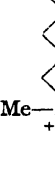
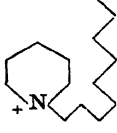
With cationic detergents it would be expected that ionic forces and ion-dipole association would also be operative, the former becoming increasingly effective at higher pH values, and the latter at all pH values on the alkaline side of the isoelectric point. With cetyl pyridinium bromide and cetyl trimethylammonium bromide, however, at pH 12.5 the binding is equivalent to 1.7 and 1.3 mmole/g. respectively and is independent of the presence of inorganic electrolyte. This is only slightly in excess of the total anionic side chains of the protein (1.24 mmole/g.<sup>11</sup>) and can readily be accounted for since some hydrolysis of the protein occurs under these conditions of pH and temperature, rendering available fresh ionized carboxyl groups which are capable for adsorbing long chain cations. There is no evidence of the binding of these long chain cations to any other site in the protein molecule. As the pH is lowered, the number of cations bound decreases and on the acid side of the isoelectric point no combination takes place at all. With cetylamine, however, at pH 11, in the presence of M NaCl, although a very precise estimate of the number of molecules bound was not possible, it was evident that between 8 and 10 mmole/g. were adsorbed, indicating some adsorption at the keto-imide backbone groups, presumably by dipole-dipole association.

It has therefore to be explained why some detergent anions and cations are capable of being adsorbed at the keto-imide groups of the main chains of the protein whereas others, under comparable conditions, are not. The most likely reason seems to be connected with the size of the amphipathic ions, particularly the cross-sectional area of the head group. The primary alkyl sulphates and the primary amine, having the smallest cross-sectional area, appear to be able to penetrate between the protein side chains and approach sufficiently closely to the keto-imide groups to enable ion-dipole association to become effective, whereas the flavianate anion and the cetyl pyridinium and cetyl trimethylammonium cations, being the largest are incapable of penetrating to these groups at all. The secondary sulphate and the alkyl naphthalene sulphonate have intermediate molecular dimensions and appear to be able to penetrate into about half of the keto-imide groups. Table I shows the dimensions of the head groups of the various detergent ions and an estimate of their penetrability into the backbone of the gelatin molecule.

This explanation is somewhat similar to that given by Schulman and Armstrong<sup>18</sup> to explain the effect of various ionic groups in haemolytic

<sup>18</sup> Schulman and Armstrong, in *Surface Chemistry* (Butterworth, London), 1949, p. 275.

TABLE I

Amphipathic Ion	End Group	Minimum Cross-section	Estimated Penetrability into the Backbone *
		(Å <sup>2</sup> )	(%)
Primary alkyl sulphate . . . . .	 . . . . .	20	100
	SO <sub>4</sub> ' . . . . .	25	
Primary alkyl amine . . . . .	 . . . . .	20	100
	NH <sub>3</sub> <sup>+</sup> . . . . .	20	
7-alkyl naphthalene 3-sulphonate . . . . .	 . . . . .	35	62
	SO <sub>3</sub> ' . . . . .	25	
Secondary alkyl sulphate . . . . .	 . . . . .	40	46
	SO <sub>4</sub> ' . . . . .	25	
Flavianate (2 : 4-dinitro 1-naphthol 7-sulphonate) . . . . .	 . . . . .	47	0
	SO <sub>3</sub> ' . . . . .	25	
Alkyl trimethylammonium . . . . .	 . . . . .	20	0
	Me-N <sup>+</sup> (Me) <sub>3</sub> . . . . .	49	
Alkyl pyridinium . . . . .	 . . . . .	72	0

\* Assuming primary sulphates and amines can penetrate to all the keto-imide groups.

and enzymatic activity. They point out that the charge centres of the  $-\text{NH}_3^+$  and  $-\text{OSO}_3^-$  groups can approach a dipole, such as the  $-\text{OH}$  of cholesterol, more closely than those of  $-\text{NMe}_3^+$  and  $-\text{SO}_3^-$  respectively. With proteins, however, the steric effect of the side chains on penetration to the backbone is probably even more important. Thus, the primary sulphate is able to penetrate more completely than the more bulky secondary sulphate.

It is concluded that two types of mechanism are responsible for the primary adsorption of amphipathic anions and cations to gelatin. First, a purely coulombic ion-ion attraction, the extent of which depends on the net charge on the protein and the number of charged groups on the amphipathic ion and is independent of the size of the ion. Secondly, an ion-dipole association between the amphipathic ions and the keto-imide groups in the backbone of the protein. This mechanism is inhibited by hydrogen ions, encouraged by inorganic electrolyte and is susceptible to the size and shape of the amphipathic ion.

My thanks are due to the Director and Council of the British Leather Manufacturers' Research Association for permission to publish these results and also to Mr. K. G. E. Wyatt for assistance with the experimental work.

*The British Leather Manufacturers' Research Association,  
London, S.E.1.*

#### GENERAL DISCUSSION\*

**Prof. F. Haurowitz** (Bloomington, Indiana, U.S.A.) said: The reaction between proteins and cationic detergents takes place slowly. When we mixed 8 mg. horse haemoglobin with 2 mg. desogen (tolyldodecyltrimethylammonium chloride) in 2 ml. of 0.05 M borate-phosphate-citrate buffer (Stenhagen-Teorell), we obtained after 30 min. 7.5 mg. precipitate containing 27 desogen molecules per haemoglobin molecule. After 24 hr. a second precipitate was formed; its amount was 0.63 mg. and the ratio desogen/haemoglobin was 324/1. The slow secondary reaction consists, apparently, of the crystallization of the detergent on the surface of the primary haemoglobin-detergent complex.

**Dr. K. G. A. Pankhurst** (London) said: Apropos Dr. Haurowitz's remarks, we have also found from viscosity studies<sup>1</sup> that the protein-detergent reaction, under conditions which we believe to involve the backbone  $-\text{NH}-\text{CO}-$  groups, takes place slowly, although coulombic binding is very rapid. This has also been observed by Joly<sup>2</sup> in his flow birefringence work. It is not to be expected that the ease of penetration to the  $-\text{NH}-\text{CO}-$  groups will be the same for all proteins since this will depend largely on the sequence of amino acid residues in the main chain and other structural features, which may account for some of the specificity to which Dr. Edsall has referred. In gelatin, the apparent inaccessibility of the  $-\text{NH}-\text{CO}-$  groups to large ions may well be due to the high proportion of proline and hydroxy-proline residues, causing frequent twists in the main chain.<sup>3</sup>

**Dr. J. H. Schulman** (Cambridge) said: Pankhurst's table giving the

\* On two preceding papers.

<sup>1</sup> Pankhurst, *Bull. Soc. Chim. biol.*, 1949, **31**, 703.

<sup>2</sup> Joly, *ibid.*, 1948, **30**, 398.

<sup>3</sup> Astbury, *J. Int. Soc. Leath. Tr. Chem.*, 1940, **24**, 69.

dimensions of the head group and hydrocarbon chains do not agree with the results obtained from surface chemical techniques. Thus the  $\text{—SO}_4^-$  polar group can be readily squeezed to  $20 \text{ \AA}^2$  as seen from  $F\text{—}A$  curves of  $\text{C}_{22}$  sulphate monolayers. The  $\text{—N}^+(\text{CH}_3)_3$  polar group can be contracted to  $31 \text{ \AA}^2$  as seen from  $F\text{—}A$  plots of  $\text{C}_{20} \text{N}(\text{CH}_3)_3\text{HCl}$  monolayers and the long-chain pyridium hydrochloride packs, with an area per molecule of possibly  $40 \text{ \AA}^2$ .

It is possible to consider an explanation for Pankhurst's results on the ease of hydration of these polar groups and also the hydrophobic-hydrophilic balance change of the protein molecule on adsorption of the compounds. This balance is changing on adsorption of the long-chain compounds and will act as a barrier to the approach of further molecules. This can be demonstrated especially with long-chain trimethylammonium salt compounds, which can adsorb on to the ionized carboxyl groups and make the protein molecule hydrophobic. This hydrophobic barrier would prevent the approach of further trimethylammonium ions to the protein molecule.

**Dr. K. G. A. Pankhurst** (*London*) said: I do not think that the areas given by Dr. Schulman, derived from monolayer measurements, are applicable in the case under discussion since considerable overlap and interlocking of the head groups may take place as the film is compressed. The areas quoted in my paper were derived from measurements of scale atom models and represent the projected area of single molecules. This is not ideal since no account is taken of hydration. It is, I think, significant that whether one takes Schulman's figures or those which I have given, the same conclusion is drawn, that under conditions where one would expect adsorption to take place at the backbone  $\text{—NH—CO—}$  groups, the larger the ion, the less is its penetrability.

The suggestion that highly hydrated ions such as  $\text{—N}^+\text{—Me}_3$  act as a hydrophobic barrier when already adsorbed at the negatively charged side-chain sites does not, I think, explain all the facts since under iso-electric conditions where there is no evidence for coulombic binding of either anions or cations, the larger ions are still incapable of penetrating to the backbone  $\text{—NH—CO—}$  groups even when inorganic salt is present, i.e. when smaller ions such as  $\text{—SO}_4^-$  and  $\text{—NH}_4^+$  are strongly adsorbed.

**Dr. R. Matalon** (*Cambridge*) said: How do the number of molecules of homologous alkyl sulphates taken up by the protein (gelatin) compare, when these detergents are used at the same concentration and below the micellar state? In other words is it possible to establish for this present study of protein-detergent association an effect similar to the Traube effect? Then it might help to explain the nature of the protein-detergent binding in the bulk state.

**Dr. K. G. A. Pankhurst** (*London*) said: In answer to Dr. Matalon, although increasing the length of hydrocarbon chain from  $\text{C}_8$  to  $\text{C}_{18}$  has no effect on the primary binding capacity of long chain sulphates, the detergent/protein ratio corresponding to the beginning of complex separation does decrease by a factor of about 1.5 to 2 per  $\text{CH}_2$  group.

**Prof. E. K. Rideal** (*London*) said: I find the problem of interaction of detergents with native proteins much more difficult to understand than the reaction with fibrous proteins. It seems clear that there are three distinct steps in this interaction, the first which occurs with extremely dilute solutions of detergents seems to be highly specific for the protein and it appears that on these proteins there are a few, say 4 or 5, readily accessible groups. If the chain is not too long this is the only reaction involved. With longer chains we get the second stage, namely, a reaction with all the available amino groups of the protein, and finally, with still longer chains and higher concentrations, we get the solubilizing adsorption which may be on the  $\text{—CO—NH}$  groups, but equally well may be the solution of the chains in the now hydrophobic protein surface, requiring

the  $-\text{SO}_3$  groups in the surrounding medium. It seems likely that stage 2 or 3 is associated with denaturation or unfolding.

**Dr. K. G. A. Pankhurst** (*London*) said: I do not think there is much evidence to suggest that the  $-\text{NH}-\text{CO}-$  groups are centres solely for solubilizing adsorption as suggested by Prof. Rideal. Rather do I think that the evidence points to their being centres for primary adsorption (with the lipophilic tails of the detergent ions outwards) in the same general fashion as the charged side chains, except that they have a different dependence on pH and inorganic salt. I think that solubilization is brought about entirely by the formation of a second layer, attached to the first by van der Waals' forces, with the ionic groups outermost.

**Prof. J. Murray Luck** (*Stanford, California*) said: The work I have reported is difficult to compare with the other studies reported this afternoon. The fatty acids we used contained less than ten carbon atoms. They were monodisperse and not micellar and it is certain that the binding of a micelle is different from the binding of single ions. With few exceptions we have restricted our studies to well-defined proteins. Gelatin we have avoided, partly because it is not well characterized, but also because it is quite unsuitable for the viscosity, thermal stability, and dialysis-equilibrium studies in which we were interested.

As far as the binding studies are concerned, the method used rests upon the establishment of a thermodynamic equilibrium between the reactants. The method does not permit the complete titration of positive groups and/or other binding centres for which it appears to be necessary to permit the formation of a precipitate and the development of a polyphase system. I am not prepared to say which method is the more meaningful in so far as biological implications are concerned: this is still a matter of mere opinion.

It is also necessary to emphasize the specificity of the phenomenon. The serum albumins alone bind the fatty acid anions; at least the binding capacity of other proteins is virtually negligible under the conditions of our experiments. This is the experience of workers in other laboratories as well.

As for dodecyl sulphate, it is well to recognize that this substance plays a dual role. In very low concentrations it is actually a good stabilizer of serum albumin, protecting it even more effectively than caprylate against urea denaturation. The viscosity-concentration curve rises sharply, however, from this point on, and the denaturing property of the detergent soon comes into evidence. Dr. Duggan found the critical point of inflection, where the stabilizing property was maximal, was at a mole ratio of detergent/protein = 9.

**Dr. M. Joly** (*Paris*) said: The interaction between protein and detergent does not necessarily involve the denaturation of the protein. For instance, if sodium dodecyl sulphate at very low concentration is added to a solution of tobacco mosaic virus, we observe by streaming birefringence a change of shape of the virus particles, but the biological properties of the virus do not change. The protein virus is not denatured by the sodium dodecylsulphate at a concentration far below the critical concentration of micelle formation.

**Dr. H. L. Booij** (*Leiden*) said: Some ten years ago Mrs. Teunissen-van-Zijp, in our laboratory, made some experiments on the influence of organic anions on the charge of a positive protein (clupein). When comparing the concentrations of fatty acid anions needed to reach the state of zero-charge, it was found that between  $\text{C}_6$  and  $\text{C}_{11}$ , the influence of the length of the carbon chain was very pronounced ( $\text{C}_6$ , 0.6 mole/l.;  $\text{C}_8$ , 0.1 mole/l.;  $\text{C}_{10}$ , 0.01 mole/l.;  $\text{C}_{12}$ , 0.003 mole/l.). As in this homologous series the interacting negative groups of the fatty acid anions and the positive groups of the protein are the same throughout, these experiments suggest the important role of the van der Waals' forces between

the carbon chains and the protein. So I quite agree with Prof. Luck that the binding capacity of proteins generally depends on Coulomb forces between oppositely charged groups and van der Waals' forces between carbon chains, and non-polar side chains of the amino acid residues of the protein. In other organic molecules (e.g. with OH groups) hydrogen bonds may be expected, too.

**Dr. J. T. Edsall** (*Harvard*) said: I should like once more to emphasize the specificity of the stabilizing action of fatty acid anions on serum albumin, which Dr. Luck has discussed. Oncley, Melin and Gross, in our laboratory, showed during the war that  $\gamma$ -globulin is not at all stabilized against heat denaturation by these reagents; in fact, it becomes somewhat less heat-stable in their presence. On the other hand, reagents such as glycine, or the salts of some dicarboxylic acids, do significantly stabilize  $\gamma$ -globulin, although the effects are far less dramatic than those of fatty acid anions on serum albumin.

It might be of interest to study the stereochemical specificity of some of these reactions with albumin. For instance, would the binding constants be different for *d*- and *l*-mandelate, or for *cis*- and *trans*-cinnamates? Considering the great importance of steric factors in immunologic reactions, one might expect to find something a little similar here.

**Dr. O. Hoffmann-Ostenhof** (*Vienna*) said: In connection with the experiments reported it may be of interest that the interaction of cation detergents with protein can be well demonstrated by measuring the inactivation of enzyme activity by these compounds. Enzymes are, as far as it is known, proteins and are rather unspecifically inhibited by cationic detergents. Experiments performed in my laboratory with various enzyme preparations (urease, papain, catalase, phosphomonoesterases) have shown that cationic detergents of various chain length ( $C_8$  to  $C_{18}$ ) have qualitatively the same effect on enzyme action; quantitatively there is a marked difference, the  $C_{18}$  compound being about two hundred times a stronger inhibitor of the action of the enzymes named than the  $C_8$  compound.

**Dr. J. A. V. Butler** (*London*) said: I would like to ask Prof. Luck whether the "bond free energies" he gives do not really refer to displacement reaction. They are based on the equilibrium of the anions between a dialysis bag containing the protein and an outer buffer solution. When an anion enters the bag it will be accompanied by an equal quantity of a positive ion, or will displace an equivalent quantity of negative ions. So what is really measured is either the sum of quantities which refer to the distribution of both positive and negative ions, or a difference representing the displacement of one anion by another. Since proteins hold water very tenaciously and it has been found that each polar side group binds at least one water molecule with a considerable energy, I would also like to ask if he thinks that the anions are bound on top of this bound water, or do they displace it?

It would be very interesting to have the bonding energies ( $\Delta H$ ) instead of the free energies which are quoted. Have any temperature coefficients been measured?

**Dr. J. T. Edsall** (*Harvard*) said: Dr. Butler asks whether any  $\Delta H$  values have been measured for these reactions. Klotz and Urquhart<sup>4</sup> reported  $\Delta H$  as  $-2100$  cal./mole for the binding of methyl orange by bovine serum albumin and as  $-2000$  cal./mole for azosulphathiazol. Still more recently, Scatchard, Scheinberg and Armstrong<sup>5</sup> have obtained  $\Delta H$  values for the binding of chloride and thiocyanate by albumin. These values are extremely low, not more than a few hundred cal./mole, and could indeed be regarded as zero within the experimental error of the measurements.

**Prof. J. Murray Luck** (*Stanford, California*) replied: I don't know how to answer Dr. Butler's questions about bond energies and water.

<sup>4</sup> *J. Amer. Chem. Soc.*, 1949, **71**, 847.

<sup>5</sup> *Ibid.*, 1950 (in press).

As for the former, I quite agree that the bond energies mentioned in the paper may not be as amenable to the precise interpretations that apply to small molecules and very simple systems. Competition of other anions derived for example from salt and buffer, must confuse things somewhat so that the bond energy is really a net value representative of the affinity between positively charged groups on the protein and the added anion, *but in the presence of certain competing anions*. It is relevant to mention that the bond energies are calculated only for the first ion that is bound. In so far as statistical factors predominate and interference with the adding of the remaining ions is negligible the calculated bond energy is probably about the same for all of the ions that are bound. There is, however, another qualification that should be made. The value is obviously statistical and presupposes that the first ion to be bound is not preferentially directed in each reacting protein molecule to a guanidine group, a lysine group, or some certain amino acid side chain.

I shall have to skip over the water question: I don't know what effect, if any, hydration of the positively charged groups would have.

**Prof. J. R. Squire** (*Birmingham*) said: As we have heard today various examples in which the body lipoprotein differs in behaviour from those studied experimentally *in vitro*, it seems appropriate to give a more encouraging example of the relevance and fundamental importance of such combinations in human economy. With Dr. C. Ricketts and Dr. E. Topley of the Medical Research Council Units at the Birmingham Accident Hospital, studies have been made of the self-sterilizing power of human skin against pathogens such as haemolytic streptococci. These organisms are destroyed by oleic acid which is found free only with other lipids in quantities corresponding to a layer 0.5 to 1.0  $\mu$  thick on the skin. As previously shown by Dubos, the bactericidal power of oleic acid is inhibited by serum albumin. We have shown that the self-sterilizing power of skin against streptococci is correspondingly inhibited by serum albumin. As described by Prof. Murray Luck, the combination is quantitative (even though in our experiments concentrations leading to micelle formation were employed) and is specific—for example, neither the keratin surface of skin nor added peptone interferes with self-sterilization. Breaches of the skin surface leading to its contamination with serum proteins would be expected to allow the multiplication of pathogenic organisms; this finding is the rule in dermatitis and other conditions. The complete understanding of lipid-protein interaction is clearly of the greatest importance to many aspects of biology and medicine.

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## STUDIES ON LIPO-PROTEIN CENAPSES OF HORSE SERUM

By M. MACHEBOEUF AND P. REBEYROTTE

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The lipoprotein obtained by acid precipitation of horse serum contains protein, lecithin and cholesterol esters, but no other lipids are present. The fraction obtained from horse serum preserved aseptically for a period of ten days, differs from that produced from a fresh serum. This indicates that ageing of the serum causes rapid structural alterations of the lipoprotein constituents.

The lipoprotein fraction obtained by acid precipitation of fresh serum is well defined and behaves homogeneously on electrophoresis. The electrical mobility observed is very close to that of crystalalbumin. When the lipoprotein fraction is treated for lipid extraction, the protein obtained is still homogeneous, but is not identical with crystalalbumin or with any of the known globulins.

Ultra-centrifugation and electron microscope studies indicate that the lipo-protein particles are of homogeneous size of the same order as those observed with the known serum proteins. Every particle must be formed of one protein molecule, or possibly two, associated with some lipid molecules. The protein-lecithin association must be of the same type as that obtained when protein is mixed with a saponium.

A lipo-protein fraction containing up to 40 % lipids but, nevertheless, water-soluble in neutral or alkaline medium ( $\text{pH} > 6.5$ ) was isolated by one of us <sup>1</sup> twenty years ago from horse serum. This fraction, termed C.A. (i.e. cenapses precipitated by acid), is studied in the present work. C.A. consists solely of protein, lecithin and cholesterol esters. The principle of C.A. precipitation is reviewed, and the structural alterations which C.A. undergoes when serum is preserved for a long time before fractionation are examined.

Further investigations have been carried out, to determine the nature of the protein present in C.A. using electrophoresis. The mobility of C.A. as well as that of the associated proteins have been observed. The size of C.A. particles has been determined using ultracentrifugation and electron microscope techniques.

Electrophoretic experiments have been carried out in collaboration with Mr. Demende. Ultracentrifugation and electron microscope studies have been performed in collaboration with P. Lepine and J. Giuntini.

**Principle of C.A. Isolation.**—Globulins contained in horse serum are precipitated with half-saturated  $(\text{NH}_4)_2\text{SO}_4$ ; the precipitate is redissolved in water and recovered by a second treatment with  $(\text{NH}_4)_2\text{SO}_4$ . The supernatant liquids obtained from these two precipitations are mixed together. They contain the albumins as well as the C.A. and are half-saturated with salt. To this solution M/10  $\text{H}_2\text{SO}_4$  is added until pH 3.9 is reached. The precipitate thus obtained is redissolved in a small volume of water and ammonia added until the pH is 7; a further precipitation is effected by readjusting the pH to 3.9 (precipitate I).

Under these conditions the concentration of ammonium sulphate is smaller, consequently only partial precipitation of the albumins takes place but precipitate I contains a large amount of lecithin and cholesterol esters. Re-dissolution of precipitate I followed by re-precipitation increases the relative proportions of lecithin and cholesterol esters in precipitate 2, whereas more albumins and lipids remain in the supernatant liquid in which the concentration of ammonium sulphate decreases. These operations (dissolving at pH 7 and precipitating at pH 3.9) when repeated several times effects a fractionation. This is demonstrated in Fig. 1 where the composition of the precipitate is studied as a function of the number of precipitations.

The composition of the precipitate remains constant after six precipitations; only slight decrease in the amount of the precipitate is observed from 6 to 9 (see Fig. 1) due to the solubility characteristics of the C.A. at pH 3.9. If the C.A. is mixed with a great excess of crystalbumin, it dissolves in a large amount of water even at pH 3.9. This explains why very small amounts of water are used for redissolving precipitate I during fractionation.

<sup>1</sup> Macheboeuf, *Bull. Soc. Chim. biol.*, 1929, **11**, 268 and 483; also *Thesis* (Paris, 1928); *Rev. Générale Colloïdes*, 1929, **7**, 352 and 393; *Actualités Sci. Ind.*, No. 448, Vol. 1 (Paris, 1936).



**Constitution of the C.A. Fraction.**—Precipitate 9 indicated in Fig. 1 contains 59.3 % protein, 22.7 % lecithin (no cephalin present), and 17.9 % cholesterol esters (no free cholesterol present). The absence of free cholesterol and other lipids in the C.A. fraction indicates the specificity of the fractionation technique.

These figures for the composition of C.A. were obtained before the war. The following experiments were undertaken in 1946 in a period when the conditions in France resulting from war damage were such that the horses were undernourished. The proportion of C.A. in the serum was then very small and it was impossible to obtain C.A. fractions as rich in lipids; for instance, the composition found was protein 83 %, lecithin 12.5 %, and cholesterol esters 4.5 %.

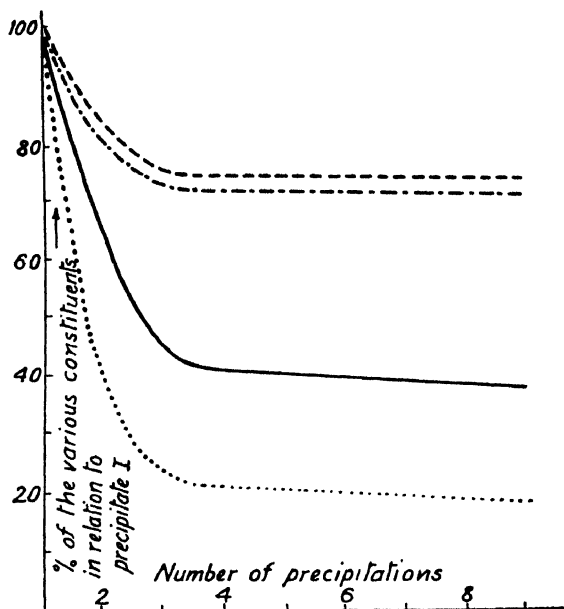


FIG. 1.

- Phospholipids
- . . . . Acids
- Cholesterol
- ..... Total Substances

**Serum Ageing.**—Previous work in our laboratory<sup>2-5</sup> has shown that when serum is preserved aseptically the lipo-proteins were altered. Thus the cholesterol of aged serum cannot be liberated by the cyclopentane-phenanthrene saponosides, while this operation is possible on fresh serum. In the present work, the influence of serum ageing on the composition of C.A. fraction is considered.

We have observed that the composition of C.A. varies with the conditions of the serum, i.e. whether the serum is fresh or has been kept aseptically for a few days in a refrigerator, or preserved at ordinary temperatures. The volume and mass of the wet precipitate obtained by the last centrifugation increases markedly when the serum has been preserved.

<sup>2</sup> Delage, *Bull. Soc. Chim. biol.*, 1936, 18, 1311; *Thesis* (Paris, 1937).

<sup>3</sup> Tayeau, *Compt. rend. Soc. Biol.*, 1939, 130, 1029 and 1944, 138, 423.

<sup>4</sup> Tayeau, *Bull. Trav. Soc. Pharm. Bordeaux*, 1945, 83, 62 and 89.

<sup>5</sup> Macheboeuf, Polonovski and Faure, *Compt. rend.*, 1948, 227, 1420.

Table I summarizes the results obtained with serum kept at 4° C under a layer of ether. This solvent prevents bacterial growth and does not dissolve any of the lipids; consequently the experimental results are practically unimpaired. In Table I the weight is expressed in g./100 ml. serum. The facts are even clearer when the separation of C.A. from serum is effected by the more rapid although less specific method of Macheboeuf and Dizerbo.<sup>6</sup> The results then obtained are summarized in Table II.

The lecithin/protein ratio in serum No. 3 decreased from 10 % to 2 % in a period of 10 days; the weight of the wet precipitate increases by 200 % whereas the increase is only 40 % for the dry C.A. It follows that rapid alterations in the structure of lipo-protein system of the serum takes place. But if a solution of C.A. obtained from fresh serum is preserved in a refrigerator its properties remain unchanged for

TABLE I

	Weight of Wet C.A.	Weight of Dry C.A.	Proteins	Cholesterol Esters	Lecithins
Serum, fresh . . . . .	4.15	1.00	0.80	0.045	0.121
Serum, preserved three days	7.85	1.44	1.30	0.065	0.083
Serum, preserved ten days .	8.20	1.33	1.12	0.077	0.062

TABLE II

	Weight of Wet C.A.	Weight of Dry C.A.	Proteins	Cholesterol Esters	Lecithin
<i>Serum No. 2</i>					
Serum, fresh . . . . .	4.9	1.00	0.84	0.034	0.067
Serum, preserved three days	8.0	1.36	1.28	0.053	0.076
Serum, preserved seven days	16.5	1.32	1.16	0.070	0.055
Serum, preserved ten days .	16.0	1.28	1.14	0.071	0.025
<i>Serum No. 3</i>					
Serum, fresh . . . . .	5.6	1.09	0.815	0.043	0.085
Serum, preserved three days	8.4	1.58	1.37	0.052	0.089
Serum, preserved seven days	16.2	1.47	1.20	0.070	0.042
Serum, preserved ten days .	16.0	1.43	1.18	0.070	0.025

many days. Thus the alterations shown in Table II are not due to spontaneous alterations of the C.A. itself, but could be due to lipid or protein exchange between C.A. and some other lipo-protein cenapse of the serum, or to the presence of an enzyme.

**Electrophoresis.**—We have shown in previous work<sup>7,8</sup> that protein and lipids in the C.A. move together in an electrical field. The apparatus then used was quite primitive. The present work has been carried out using the Tiselius apparatus. C.A. which was very rich in lipids and prepared from fresh horse serum has been compared with the crystallbumin of the same serum. The pH was maintained at 8 using M/30 phosphate buffer. The proteins obtained from C.A. have also been examined (after removing as much as possible of the lipid constituents by the method of Hardy and Gardiner<sup>9</sup> using absolute alcohol at -15° C

<sup>6</sup> Macheboeuf and Dizerbo, *Compt. rend. Soc. Biol.*, 1939, **132**, 268.

<sup>7</sup> Macheboeuf and Vanaud, *ibid.*, 1941, **136**, 1249.

<sup>8</sup> Macheboeuf, Delsal, Lepine and Giuntini, *Ann. Inst. Pasteur*, 1943, **69**, 321.

<sup>9</sup> Hardy and Gardiner, *J. Physiol.*, 1910, **40**, 68.

and then cold ether. This method<sup>9</sup> extracts the totality of cholesterol esters and approximately half the lecithin contained in the C.A.).

The diagrams of Fig. 2 to 6 correspond to the falling boundaries observed after 4500 sec. under a potential gradient of 5 V/cm. Fig. 2 and Fig. 3 show that C.A. is practically as homogeneous as crystalalbumin, Fig. 4. A slight dissymmetry in the graph suggests the existence of a slow-moving fraction in a very small proportion. The protein obtained from C.A. after lipid extraction (Fig. 5) shows a single boundary and the diagram is symmetrical.

The mobilities at pH = 8 are :  $0.76 \times 10^{-4}$  cm.<sup>2</sup> sec.<sup>-1</sup> volt<sup>-1</sup> for C.A.,  $0.85 \times 10^{-4}$  for albumins and  $0.75 \times 10^{-4}$  for C.A. after lipid extraction. (The mobility of crystalalbumin remains unchanged after a treatment for lipid extraction by the method of Hardy and Gardiner). The mobilities of C.A. and crystalalbumin are very close to one another. This explains the fact that electrophoretic measurements on a serum do not usually allow the detection of the existence of C.A., the proportion of which is very small compared with the total albumins. (Fig. 6 correspond to the original serum before C.A. is extracted.) Nevertheless, it has been shown recently<sup>10</sup> that if electrophoresis of the serum is continued for a long time, it is possible to detect evidence of heterogeneity of the albumins in the serum. The mobility of C.A. after lipid extraction ( $0.78 \times 10^{-4}$  cm. sec.<sup>-1</sup> volt<sup>-1</sup>) does not differ appreciably from that of the original C.A. ( $0.75 \times 10^{-4}$ ). Hence extraction of the lipids by the method of Hardy and Gardiner does not alter the mobilities.

It appears, therefore, that C.A. is a well-defined fraction which behaves as if it were practically homogeneous on electrophoresis and that its mobility at pH 8 is only slightly less than that of crystalalbumin. The protein obtained after lipid extraction is also homogeneous.

**Nature of the Proteins contained in C.A.**—Electrophoresis has shown that the mobility of the protein extracted from C.A. is not exactly the same as that of crystalalbumin. The protein is, nevertheless, not identical with  $\alpha$ -globulin as its mobility is too high.

Work in progress with E. Barbu confirms that the protein of C.A. obtained after lipid extraction differs from crystalalbumin and globulin. Indeed, its gelling diagram<sup>11</sup> does not correspond to that of crystalalbumin nor serum globulins even after these proteins have been submitted to the lipid extraction method.

We have therefore studied the solubility of the C.A. proteins. Curves in Fig. 7 show the precipitation by  $(\text{NH}_4)_2\text{SO}_4$  at pH = 7. The solutions in each case contained 90 mg. proteins. The C.A., as well as the proteins isolated from C.A. by lipid extraction, behave as homogeneous compounds and the curves obtained are very different from those given by globulins or crystalalbumin.

In order to emphasize the difference between crystalalbumin and C.A. proteins we have tried to crystallize the proteins isolated from C.A. after lipid extraction. This protein did not crystallize when submitted to the treatment which led to perfect crystallization of the serum albumins (the crystalalbumin was submitted to the treatment of Hardy and Gardiner before crystallization). The presence of a germ of crystalalbumin in the C.A. protein did not promote crystallization.

Thus the protein associated with lecithin and cholesterol esters and present in the C.A. is not crystalalbumin nor any of the known globulins.

**Dimension of C.A. Particles.**—(1) ULTRACENTRIFUGATION.—We have found previously<sup>8</sup> that the sedimentation constant is  $2.5 \times 10^{-13}$  to  $4 \times 10^{-13}$  cm. sec.<sup>-1</sup> dyne<sup>-1</sup>. We ignored the density of the C.A. particles which differed naturally from that of the C.A. protein due to the lipids contained in these particles. The Elford formula has enabled us to

<sup>10</sup> Hoch and Hoch-Ligeti, *Biochem. J.*, 1948, **43**, 556.

<sup>11</sup> Barbu and Macheboeuf, *Ann. Inst. Pasteur*, 1948, **74**, 300; **75**, 56, 226, 426; *Bull. Soc. Chim. biol.*, 1948, **30**, 553; *Compt. rend Soc. Biol.*, 1948, **142**, 123.

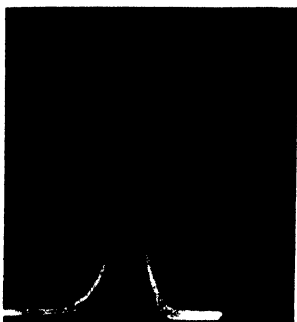


FIG. 2

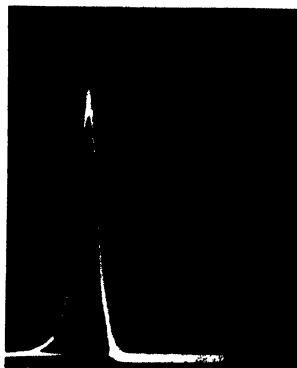


FIG. 3

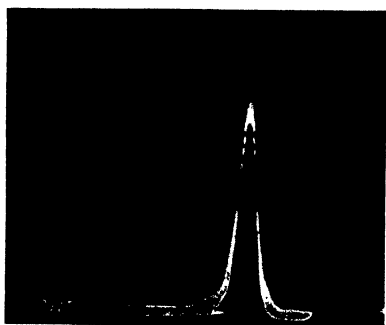


FIG. 4

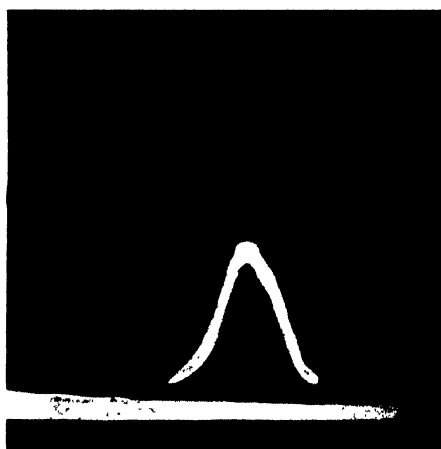


FIG. 5

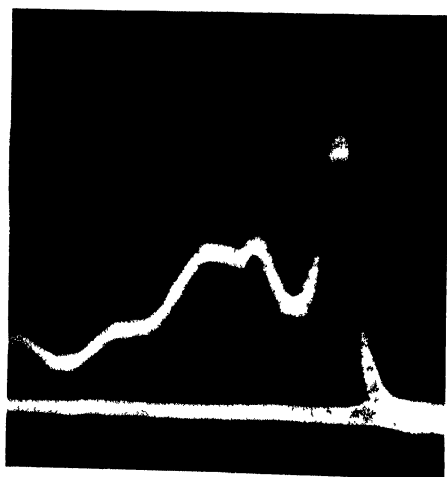


FIG. 6

FIG. 8.—A thin layer of a  $1/10,000$  solution of C.A. in distilled water is deposited on the membrane and submitted to evaporation at room temperature. The plate was obtained using 80 kV after shadowing with gold in vacuum. The magnification is 60,000. The amount of C.A. is relatively high. The particles are in some places super-posed in several layers. Measurements of the diameters give values between 6 and 10  $m\mu$ . A round, relatively large, isolated particle (probably impurity) is visible on the right just above the centre of the plate. Its diameter (22  $m\mu$ ) is useful for comparison.

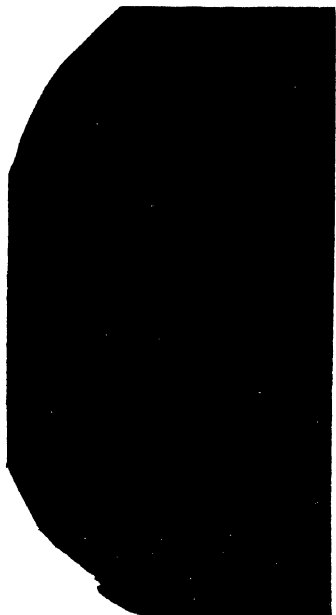


FIG. 9. A droplet of very dilute C.A. solution has been deposited, and the excess liquid removed by a capillary pipette thus leaving minute quantity of liquid. The quantity of C.A. is just greater than that necessary to produce a film at the interface. On spreading, the proteins lose their globular state; only a non-uniform thickness of the layer, probably due to the state of the lipo-protein film is visible. Some isolated particles have been deposited on this layer. Their diameter is between 5 and 10  $m\mu$ .



FIG. 10.— Shows tobacco virus taken under the same conditions as in Fig. 8 and 9. The magnification is 60,000, the diameter of the virus is known to be  $15\text{ m}\mu$ . This is used for comparison with the dimensions of the C.A. particles.



calculate the approximate value of the equivalent diameter of the particles. Such experiments are now being carried out using heavy water in order to approximate more closely to the density of the particles; more details will soon be available. Nevertheless, we can conclude that the experimental results point to the existence of a particle with dimensions of the same order as found for the normal serum proteins.

The C.A. therefore does not consist of lipids coated with proteins, but is in fact a dispersion at the molecular stage of a protein-lipid combination.

(2) ELECTRON MICROSCOPE.—The photographs (Fig. 8, 9 and 10) were obtained with a magnification of 60,000. No large droplets are visible and all particles are similar, their diameter being a few  $\mu$ . This agrees well with the dimensions calculated from sedimentation experiments and confirms that C.A. does not consist of voluminous lipid and protein aggregates. Each particle must be formed by one, or at the most two, protein molecules associated with a few lipid molecules. If the unknown

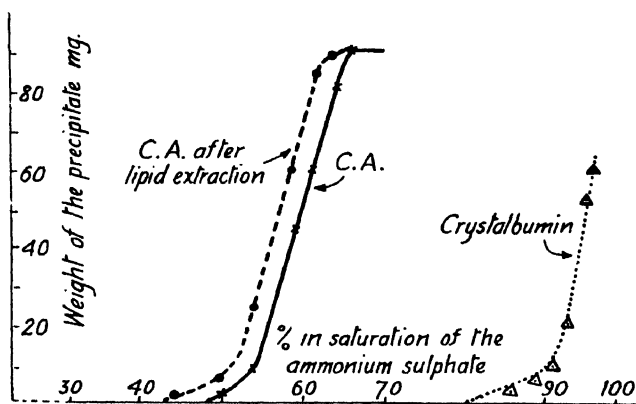


FIG. 7.

Curves of solubility as function of  $(\text{NH}_4)_2\text{SO}_4$  concentration

————— C.A.  
 ----- C.A. after lipid extraction  
 ..... crystalbumin

molecular weight of the C.A. protein is close to that of albumin, then a composition of 83 % protein, 12.5 % lecithin, and 4.5 % cholesterol esters would indicate that one protein molecule is associated with 13 or 14 lecithin molecules, and 5 or 6 cholesterol esters. The molecular weight thus deduced for the particle would be of the order of 85,000.

We have shown<sup>12</sup> that proteins associate *in vitro* with saponiums which are constituted of one or two paraffin chains and one quaternary nitrogen are stable on electrophoresis. Thus C.A. is a particular case of such an association, as lecithin contains two hydrocarbon chains and one quaternary nitrogen. Furthermore, protein-saponium compounds form water-soluble complexes with cholesterol esters. This accounts for the different ratios of cholesterol esters in the C.A. extracted from different horse serum. But the protein associated with lecithin and cholesterol esters in C.A. is a particular protein, which differs from crystalbumin and globulins and which has a marked affinity for lecithin. Its electrophoretic mobility is close to that of crystalbumin while its solubility indicates a behaviour similar to that shown by the globulins.

<sup>12</sup> Macheboeuf and Polonovski, *Ann. Inst. Pasteur*, 1948, **74**, 196, 203; Polonovski, *Thesis* (Paris, 1949), *Bull. Soc. Chim. biol.* 1948 (in press).



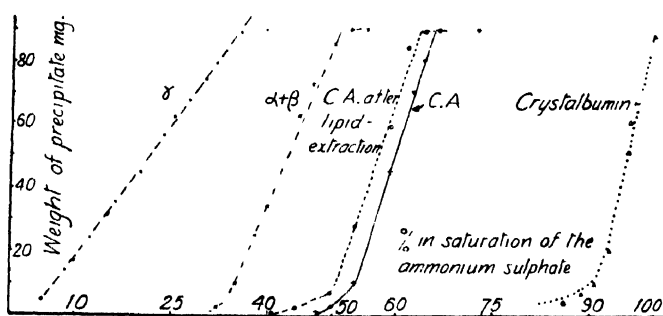
## ADDENDUM

Since the submission of our paper, we have continued our researches and I should like to add the following results.

We have repeated the experiments corresponding to Fig. 7, employing in addition, different solutions of globulins obtained from the same horse serum, and also treated by the delipidation method of Hardy and Gardiner. The curves obtained for the different globulin fractions are very different from the curve of defatted C.A. (Fig. 7*b*).

We may therefore conclude that the protein obtained on defatting the C.A. is neither crystalbumin nor any known  $\alpha$ ,  $\beta$  or  $\gamma$  globulin. It does not occur in high concentration in the plasma and its electrophoretic mobility does not allow it to be easily differentiated from crystalbumin. In addition, ammonium sulphate at 50 % saturation does not precipitate it quantitatively. These facts explained why it has not been observed before. We were able to isolate it due to its special properties when bound to lecithin and cholesterol esters.

It is to be emphasized that the defatting of C.A. by the method of MacFarlane or by the method of Hardy and Gardiner is not absolutely complete. A small proportion of phospholipid always remains (less than 10 % of the total lipid phosphorus).

FIG. 7*b*.

We have also completed the centrifugation experiments in mixtures of ordinary and heavy water. Unfortunately the centrifuge utilized (Henriot and Huguenard model) has no optical recording device. We were therefore obliged to follow the sedimentation by chemical analyses. We analyzed for proteins, cholesterol and lipid-phosphorus; the results were in good agreement within the limits of experimental error for such micro-determinations. The particle density thus calculated is  $1.10 \pm 0.05$  g. cm.<sup>-3</sup>.

When working on high concentrations of heavy water (90 %) the sedimentation of cenapses is extremely slow because the particle density is hardly greater than that of the surrounding liquid. In dealing with a mixture of C.A. and ordinary proteins one would see the lipid-free proteins sediment much more quickly than the cenapse.

Having thus obtained an approximate value for the C.A. particle density, we then calculated the diameter of a spherical particle of the same density which would sediment in ordinary water with the same speed as C.A. The equivalent diameter thus calculated was  $6.5 \pm 1.5$  m $\mu$ . This figure is in good agreement with the dimensions found by our electron microscope measurements (between 6 and 10 m $\mu$ ).

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## GENERAL DISCUSSION\*

**Dr. J. T. Edsall** (*Harvard*) said : In the studies which Prof. Macheboeuf has described on the sedimentation of lipo-protein in heavy water, with the aim of determining the density of the protein, I presume one must take into account the deuterium exchange reactions between the water and the protein itself. The hydrogens of the carboxyl, hydroxyl, amino, and peptide groups must exchange very rapidly with the deuterium of the heavy water. Hence, as the medium becomes denser by increasing the deuterium content, the protein itself also becomes slightly denser. What is the best way of evaluating this effect ?

**Prof. E. K. Rideal** (*London*) said : Is anything known about the hydrogens exchangeable with deuterium in proteins, especially in their native form ? Those on the amine groups and free carboxyl are evidently exchangeable. We might anticipate that the hydrogen in the  $\text{—CO—NH—}$  groups would exchange. Hydrogens binding the folds of the protein might exchange much more slowly.

**Prof. M. Macheboeuf** (*Paris*) said : The exchange between heavy water and protein hydrogen certainly occurs and changes the density of the protein. But the hydrogen percentage of proteins is not greater than 7.5 (including water of hydration). If all the hydrogen were substituted by deuterium, the change in the density would be approximately 7.5 %. But the exchange only takes place with some of the hydrogen atoms. The error must therefore be less than 5 %. It is on account of this fact that I proposed  $1.10 \pm 0.05$  for the C.A. density.

The method of centrifugation in mixtures of heavy water and ordinary water affords us a sufficient approximation for our purpose. Centrifugation in saline solutions of known densities would seem to have greater disadvantages because

(i) the hydration of lipo-protein particles and perhaps also their structure, may be altered ;

(ii) of the heavy metal salts, which it has been proposed, form complexes<sup>1</sup> with proteins and so change the particle density by fixation of numerous metal atoms on each particle.

**Dr. A. S. McFarlane** (*London*) said : What proportion of the total lipids of horse serum is represented in the C.A. protein ?

**Prof. M. Macheboeuf** (*Paris*) said : The method of fractionation which gives C.A. is not quantitative since the C.A. are slightly soluble under the experimental conditions used. Nevertheless one can estimate the concentration of C.A. as roughly 2.5 g./l. of horse serum. The quantity of lipids in the C.A. would hence be of the order of 1 g./l.

**Prof. E. Chargaff** (*New York*) said : Is it not possible that your partially defatted lipo-protein differs in solubility from, say,  $\beta$ -globulin because of the lipids still retained in it ? And have you carried out any immunization experiments with your lipo-protein ?

**Prof. M. Macheboeuf** (*Paris*) said : We are now carrying out immunization experiments with the C.A. but they are not as yet completed. As I mentioned in my paper, the defatting of the C.A. proteins is never strictly quantitative whether one chooses the method of McFarlane or the method of Hardy and Gardiner. A small proportion of phospholipids always remains in the protein. But this is a general fact ;  $\alpha$ - and  $\beta$ -globulins defatted by the same methods also retain some lipid. It is therefore impossible to answer Prof. Chargaff's question, since there is no strictly quantitative defatting method which does not denature proteins. It is to be remarked, nevertheless, that the removal of 90 % of the C.A. lipids does not appreciably change their solubility in ammonium sulphate solutions (see Fig. 1). The removal of the remaining 10 %

\* On preceding paper.

<sup>1</sup> Macheboeuf and Viscontini, *Ann. Inst. Pasteur*, 1945, **71**, 188 ; 1946, **72**, 631 ; 1946, **72**, 638.

would have to change the solubility considerably more in order to make the curve approach that of  $\alpha$ - and  $\beta$ -globulins.

**Dr. A. Lasnitzki** (*Birmingham*) said: May I ask Prof. Macheboeuf whether the lipo-protein complex he has isolated bears any relation to the thromboplastic protein of the blood and whether the ash of that substance shows the presence of calcium.

**Prof. M. Macheboeuf** (*Paris*) said: We have not as yet looked for a relation between the horse C.A. and the thromboplastic fraction; we have not found any calcium in the C.A.

**Prof. J. Murray Luck** (*Stanford, California*) said: What is it that really happens in the ageing of plasma, to which Prof. Macheboeuf has also referred? Presumably the proteins would not be rendered unhappy by the water itself. Is it that oxidation goes on, which, through conversion of  $-SH$  groups to  $-S-S-$  might reasonably be expected to alter the solubilities of the proteins? Have experiments been conducted to investigate the influence of anaerobiosis on the ageing phenomenon. Also there is the question of proteolytic enzymes. For most tissue extracts it is reasonably certain that the presence of proteolytic enzymes is responsible for some undesired changes in the proteins under study. Are the proteolytic enzymes of plasma a similar source of trouble? May they not contribute to the change in protein proportions observed in plasma which is not strictly fresh?

**Prof. M. Macheboeuf** (*Paris*) said: It is highly probable that enzymes play an important role in the modifications which lipo-proteins undergo during the ageing of plasma. I have no definite proof of this opinion, but I shall cite the following observation. When horse serum is stored for about ten days under sterile conditions, the C.A. which may be extracted are quite different from those obtained from the fresh serum. Nevertheless, the C.A. obtained from fresh serum and purified as much as possible can be kept for a very long time without any apparent change.

Last year, I was still in possession of two ampoules in which I had sealed C.A. solutions 20 years ago. They were still clear. I opened one a year ago and an examination of the contents showed no appreciable change. The isolated C.A. are, therefore, remarkably stable. This is probably due to the elimination of enzymes during the purification. Unfortunately we cannot as yet affirm the complete identity of a protein fraction obtained by fractionation and the protein pre-existent in the plasma. Hence the C.A. obtained are possibly more stable than the cenapses originally present in the plasma.

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## THE LIPO-PROTEINS OF HUMAN PLASMA

By F. R. N. GURD, J. L. ONCLEY, J. T. EDSALL AND E. J. COHN

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The lipids of fasting human plasma exist almost exclusively in the form of two distinct types of lipo-proteins, having electrophoretic mobilities of  $\alpha_1$ - and  $\beta_1$ -globulins respectively. The solubility properties of these plasma lipo-proteins are those of typical proteins. The  $\alpha$ -lipo-proteins represent about 3 % of the total plasma proteins, contain about 35 % lipid, and have a molecular weight near 200,000; the  $\beta$ -lipo-proteins represent 5 % of the total plasma proteins, contain 75 % lipid, and have a molecular weight of 1,300,000. Both lipo-proteins contain phospholipids, unesterified cholesterol, and cholesterol esterified with fatty acids, and the  $\beta$ -lipo-proteins also bind much of the carotenoids and oestrogens present in plasma.

THE LIPIDS OF NORMAL HUMAN PLASMA do not exist, in any appreciable concentration, in the free state but are combined with specific

proteins as well-defined lipo-proteins. When the large-scale fractionation of human plasma, at low ionic strength, was first developed in ethanol-water mixtures at low temperature<sup>1</sup> it had been expected that many of the plasma lipids would remain in the residual ethanol-water mixture from which the proteins had been precipitated. The lipids in the large pools of Red Cross plasma that were fractionated were, however, found in two readily separated fractions combined with two quite distinct types of lipo-proteins.

THE DIFFERENT LIPO-PROTEINS differ greatly in their solubility in water and ethanol-water mixtures, in their molecular size and shape, in their lipid content, and in their electrostatic interactions. The one group moves in the electric field with mobilities characteristic of the so-called  $\alpha_1$ , the other of the  $\beta_1$  serum globulins. Unlike most other protein components of plasma, these lipo-proteins cannot be frozen or dried from the frozen state without gross denaturation. Indeed, lipo-proteins appear to be susceptible to many types of denaturation not common to other plasma proteins. Only with the development of our new method of plasma fractionation<sup>2</sup> which depends upon protein-protein interactions, and in which fractional extraction has largely replaced fractional precipitation, have the  $\alpha$ -lipo-proteins been separated in a state approximating that which obtains in nature.

THE  $\beta$ -LIPO-PROTEINS are estimated to represent approximately 5 % by weight of all the plasma proteins.<sup>3</sup> They contain approximately three-quarters of the lipid of fasting normal human plasma. The  $\beta$ -lipo-proteins are euglobulins, that is to say, they are insoluble in water in the isoelectric condition in the absence of salt. Small amounts of salt have a great influence upon solubility in the region of the solubility minimum, near pH 5.4. It was as a result of solubility changes of this kind that Mellanby<sup>4</sup> was able correctly to deduce the law of ionic strength long before it was deduced from the study of simple electrolytes.<sup>5</sup>

The solubility behaviour of the  $\beta$ -lipo-proteins of human plasma is greatly influenced by their tendency to interact strongly with other proteins to form insoluble complexes. Thus, they combine with  $\gamma$ -globulins over a pH range in which the  $\gamma$ -globulins are very soluble as cations and in which the  $\beta$ -lipo-proteins are very soluble as anions. On mixing, a hundred-fold reduction in solubility has been observed. In our new method of plasma fractionation<sup>2</sup> advantage is taken of this interaction to effect quantitative separation of the albumins from the  $\gamma$ -globulins by employing conditions under which each would be soluble, but under which the  $\gamma$ -globulins are rendered insoluble by complex formation with the  $\beta$ -lipo-proteins. These complexes are subsequently decomposed, in the new process, by the addition of a high concentration of the dipolar ion glycine.

The effect of ionic strength is most marked on the solubility of the complexes formed between  $\beta$ -lipo-proteins and  $\gamma$ -globulins. Solubility increases of more than ten-fold have been observed on changing the ionic strength from 0.01 to 0.02 in phosphate buffers at pH 6.6.

<sup>1</sup> Cohn, Strong, Hughes, Jr., Mulford, Ashworth, Melin and Taylor, *J. Amer. Chem. Soc.*, 1946, **68**, 459.

<sup>2</sup> Cohn, including Mittelman (1946), Mouton, Uroma, Liu (1947), Surgenor, Barnes, Lever (1948), Gurd, Gillespie, Brown, Kahnt and others (1949) (in preparation).

<sup>3</sup> Cohn, *Experientia*, 1947, **3**, 125.

<sup>4</sup> Mellanby, *J. Physiol.*, 1905, **33**, 338.

<sup>5</sup> Lewis and Randall, *J. Amer. Chem. Soc.*, 1921, **43**, 1112.

Some, at least, of the plasma euglobulins studied by earlier workers may well have been complexes with lipo-proteins. Early observations<sup>6, 7</sup> suggested that the point of minimum solubility of certain plasma euglobulins of various species were in the neighbourhood of pH 5.5. Hardy<sup>8</sup> had noticed, in 1905, that "Globulins contain phosphorus, two analyses by Carius' method giving 0.07 and 0.08 % by weight." He added that a characteristic property of serum globulins "was their phosphorus content and the close association of the characteristic insolubility of the globulin with the presence of this element." Haslam, pursuing these investigations further, confirmed the presence of phosphorus in euglobulins and concluded that it was absent in pseudo-globulins. "Globulin contains, or is closely associated with phosphorus, rather more than 0.1 P % being found. About half this belongs to a fatty, lecithin-like body which amounts to some 8-10 % of the globulin freed from pseudo-globulin. Apparently no part of this body is detached from globulin through prolonged treatment with acids, alkalis, or salts."<sup>9</sup>

There is a close relationship between the  $\beta$ -lipo-proteins and the X-protein of Pedersen.<sup>10</sup> Pedersen observed great variations in the apparent proportions of the X-protein in his fractions, in sedimentation experiments carried out at varying concentrations of total protein. From the apparent variability of this component in solutions of differing total protein concentration, he was led to suggest that the X-protein existed in an equilibrium with other albumin and globulin components. He effected some separation of the  $\beta$ -lipo-protein of human plasma by sedimentation in solutions of high density in which it rises during centrifugation. Pedersen also pointed out that there is no lipo-protein of closely similar physical and chemical properties which has yet been recognized in any animal plasma.\*

The starting material for the preparation of the  $\beta$ -lipo-protein has, in the past, been Fraction III — O derived from Fraction II + III of human plasma.<sup>11</sup> The conditions of this separation may be briefly summarized. After the major portion of the fibrinogen and some other components have been removed in Fraction I, Fraction II + III is precipitated at pH near 7 and ethanol 0.091 mole fraction at — 5° C. Fraction III — O, containing the  $\beta$ -lipo-protein as its chief constituent, was then extracted at pH between 7.2 and 7.6 with ethanol 0.070 mole fraction at very low ionic strength (about 0.005). The  $\beta$ -lipo-protein is soluble in this medium, whereas prothrombin, the  $\gamma$ -globulins, the *iso*-agglutinins, and other components of this fraction remain insoluble. The extracted lipo-protein components of Fraction III — O could again

<sup>6</sup> Rona and Michaelis, *Biochem. Z.*, 1910, **28**, 193.

<sup>7</sup> Chick, *Biochem. J.*, 1913, **7**, 318.

<sup>8</sup> Hardy, *J. Physiol.*, 1905, **33**, 251.

<sup>9</sup> Haslam, *Biochem. J.*, 1913, **7**, 492.

<sup>10</sup> Pedersen, *Ultracentrifugal Studies on Serum and Serum Proteins* (Uppsala, 1945).

\* Johnston and Ogston<sup>12</sup> have explained these results as due to a boundary anomaly caused by changes in the concentration of a slower component due to differences in its rates of sedimentation in the presence and absence of a faster component. Their interpretation is supported by ultracentrifuge studies in this laboratory which indicate the  $\beta$ -lipo-protein to be a distinct component, and eliminate any need for postulating reversible combination with albumin or other globulins under the conditions of the ultracentrifuge experiments.

<sup>11</sup> Oncley, Melin, Richert, Cameron and Gross, Jr., *J. Amer. Chem. Soc.*, 1949, **71**, 541.

<sup>12</sup> Johnston and Ogston, *Trans. Faraday Soc.*, 1946, **42**, 789.

be precipitated by adjusting to pH 5.6-5.9 at ethanol 0.091 mole fraction and low ionic strength. Further purification was most readily effected by differential centrifugation. The principle of the method, developed by Oncley, Melin and Gross, is to separate the low density  $\beta$ -lipo-protein from contaminating denser proteins by ultracentrifugation in a sufficiently dense medium so that the  $\beta$ -lipo-protein is caused to rise slowly towards the surface.  $\beta$ -lipo-protein prepared in this way was monodisperse in the ultracentrifuge.

From analyses of three separate preparations of  $\beta$ -lipo-protein the following approximate composition was calculated: protein, 25 %; phospholipid, 30 %; cholesterol (free + esters), 45 %. Nearly all the carotenoid of plasma is bound to the  $\beta$ -lipo-protein.\* The absolute amount of carotenoid present is of the order of 0.02-0.03 % and is, therefore, extremely low (on the average less than 1 mole of carotenoid per mole of protein). At least one of the estrogens of plasma, estriol, has been estimated to be combined specifically with the  $\beta$ -lipo-protein<sup>13</sup> and not with the  $\alpha$ -lipo-protein.

The  $\beta$ -lipo-proteins are very large molecules of approximately spherical shape. The partial specific volume of the anhydrous protein is approximately 0.950<sup>14</sup> and that of the hydrated protein approximately 0.97.<sup>10</sup> Therefore, the sedimentation rate of this protein in the ultracentrifuge is extremely sensitive to the density of the medium. The intrinsic viscosity is 0.041.<sup>14</sup> This figure, combined with the difference in partial specific volume between the anhydrous and the hydrated protein, would indicate that the molecule is approximately spherical and that the hydration is of the order of 0.6 g./g. of anhydrous protein.<sup>14</sup> Assuming the molecule to be spherical, a value of 1,300,000 is calculated for the anhydrous molecular weight. The diameter of the hydrated molecule, considered as a sphere, is 185 Å.

The biological significance of the  $\beta$ -lipo-protein as an agent of transport and exchange for lipids in their passage between the blood and tissues would appear to be very great. Experiments, using lipids or lipid derivatives containing suitable isotopes, are being carried forward in this laboratory with the aim of studying the rates of exchange and distribution in some of these reactions.

THE  $\alpha$ -LIPO-PROTEINS of human plasma have markedly different properties from the  $\beta$ -lipo-proteins. These  $\alpha$ -lipo-proteins have some similarities with the lipo-proteins isolated by Macheboeuf<sup>15, 16</sup> from horse serum in that both are soluble in water in the absence of salts and have a high electrophoretic mobility.<sup>†17</sup> However, Macheboeuf's lipo-protein contained about 50 % lipid compared with 35 % lipid in the  $\alpha$ -lipo-protein we have prepared from human plasma.

In the earlier studies carried out in this laboratory the  $\alpha$ -lipo-proteins were separated in Fraction IV — 1 : 1 that is, at ethanol 0.062 mole fraction at  $-5^{\circ}\text{C}$  and pH 5.2. They remained soluble under the conditions in which the  $\beta$ -lipo-proteins precipitated in Fraction II + III.

\* The first studies on plasma carotenoids in our laboratory were carried out by Dr. John W. Mehl in the summer of 1944.

<sup>13</sup> Roberts and Szego, *Endocrinology*, 1946, **39**, 183.

<sup>14</sup> Oncley, Scatchard and Brown, *J. Physic. Chem.*, 1947, **51**, 184.

<sup>15</sup> Macheboeuf, *Bull. Soc. Chim. biol.*, 1929, **11**, 268.

<sup>16</sup> Macheboeuf, *Etat des Lipides dans la Matière Vivante* (Paris, 1937).

<sup>17</sup> Macheboeuf, Delsal, Lepine and Giuntini, *Ann. Inst. Pasteur*, 1943, **69**, 321.

† The electrophoretic behaviour of Macheboeuf's lipo-protein preparation was somewhat complicated, and it is difficult to establish the exact electrophoretic relationship between it and the human plasma  $\alpha$ -lipo-proteins.

In the new system they remain in solution with the albumins in Fraction *a*: that is, at ethanol 0.066 mole fraction at  $-5^{\circ}\text{C}$  at pH 5.8.<sup>2</sup>

The physico-chemical data on this protein refer almost entirely to the preparations obtained by the earlier method. This lipo-protein represents approximately 3 % of the total protein of plasma.<sup>3</sup> Approximately 65 % of the dry weight consists of amino acid residues and 35 % is lipid material. The molecular weight has been tentatively estimated as 200,000.<sup>14</sup> The intrinsic viscosity, 0.066, indicated a more asymmetrical molecule than the  $\beta$ -lipo-proteins.<sup>14</sup> Oncley, Scatchard and Brown<sup>14</sup> estimated the dimensions of the molecule, considered as an ellipsoid of revolution, as 300 Å in length and 50 Å in cross-section. Presumably the  $\alpha$ -lipo-proteins function also as agents of transport and exchange for certain lipids, but the nature of their specific functions in plasma is still being investigated.

THE SOLUBILITY PROPERTIES OF THE PLASMA LIPO-PROTEINS are those of typical proteins. The response to variations in pH, ionic strength, and concentration of glycine or of ethanol are characteristic of those of simple proteins, and show no influence of the lipid moiety. On denaturation, however, the bond between the lipids and the amino acid residues is broken and the separated lipids display their characteristic insolubility in water or salt solutions.

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## STATE OF LIPIDS IN BLOOD PLASMA

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The bearing of recent work on current conceptions of the manner in which lipids and proteins are associated in plasma is discussed. Attention is directed particularly to some inconsistencies in the properties of the lipo-proteins which have been described. The densities and micellar or molecular weights do not appear to be compatible with the presence of a stabilizing protein layer which is implied by electrophoretic data.

The possibility is discussed that the association of lipids and proteins is of the same nature at all levels of particle size in plasma.

The production of artificial emulsions of lipids in serum is described and these are shown to have properties significantly different from the natural ones.

Our knowledge of this subject is largely based on a few important observations which merit recapitulation.

Horse serum from which the globulins have been removed by half-saturation with ammonium sulphate gives a further precipitate when the pH is adjusted to 3.8. After purification this material has an approximately constant composition, including 18 % cholesterol and 22 % phospholipid in addition to protein.<sup>1</sup> Lipids are also associated with all the electrophoretic fractions of human serum in amount

<sup>1</sup> Macheboeuf, *Bull. Soc. Chim. biol.*, 1929, 11, 268.

equivalent to at least a one-to-one molecular ratio between protein and lipid. However, the  $\beta$ -globulin fraction is much richer than the others, containing 8.6 % cholesterol and 10 % phospholipid.<sup>2</sup> Large  $\beta$ -globulin fractions which occur in nephrosis and jaundice are much reduced by ether extraction,<sup>3</sup> and a similar effect is observed in normal human serum if the ether extraction is accompanied by freezing to low temperatures.<sup>4</sup> The precipitate which forms in human serum at pH 7 and between 50 % and 60 % saturation with ammonium sulphate has a density of only 1.10. When purified by reprecipitation this material is almost homogeneous by electrophoresis and contains 8.5 % phospholipid, 16.4 % cholesterol and 20.4 % fatty acids.<sup>5</sup> Two lipoproteins containing 35 % and 75 % lipids have been isolated by low-temperature alcohol fractionation of human plasma and shown to be related to the  $\alpha_1$  and  $\beta_1$  globulins.<sup>6</sup>

Human serum, by contrast with a number of animal sera, shows an asymmetrical albumin boundary in the ultracentrifuge, suggesting the presence of a second or so-called X-protein sedimenting close to the albumin.<sup>7</sup> This component can be made to move more slowly than the albumin, or in the opposite direction, by increasing the density of the medium with salt, thus establishing a link with the plasma lipids. The X-component can be isolated in small quantities by high-speed centrifugation in the presence of high salt concentrations.<sup>8</sup> Since electrophoretically isolated  $\beta + \gamma$  globulin fractions contain a similar component of density 1.03 and pure  $\gamma$ -globulin fractions do not, it is established that the X-protein of the ultracentrifuge diagram is related to the  $\beta$ -globulin of the electrophoresis one.<sup>9</sup>

In the present paper these observations are discussed more critically and some new data presented.

**1. Lipid Partition in Plasma.**—Only in the case of human plasma are anything like complete data available. In this plasma only small proportions of the total lipids are present in the lipoprotein fractions precipitable between 50 % and 60 % saturation with ammonium sulphate, or by adjusting the pH to 3.8 at half-saturation with ammonium sulphate. The  $\alpha$ - and  $\beta$ -globulins isolated by electrophoresis on the other hand together contain approximately 75 % of the plasma cholesterol and 85 % of the phospholipids,<sup>2</sup> a result which is confirmed for cholesterol only in the case of the same globulins isolated by alcohol fractionation.<sup>10</sup> There is some uncertainty as to the proportion of the total lipids represented by the lipoproteins of the Harvard workers. Cohn gives a total of 3.1 g. lipids per litre in the form of  $\alpha_1$  and  $\beta_1$  globulins,<sup>11</sup> while Oncley estimates 2.2 g. lipids in the same form.<sup>6</sup> It is not clear that the latter quantities have been isolated as distinct from estimated in lipoprotein combination.

<sup>2</sup> Blix, Tiselius and Svensson, *J. Biol. Chem.*, 1941, **137**, 485.

<sup>3</sup> Longsworth, Shedlovsky and MacInnes, *J. Expt. Med.*, 1939, **70**, 399.

<sup>4</sup> McFarlane, *Nature*, 1942, **149**, 439.

<sup>5</sup> Adair and Adair, *J. Physiol.*, 1943, **102**, 17P.

<sup>6</sup> Oncley, Scatchard and Brown, *J. Physic. Chem.*, 1947, **51**, 184.

<sup>7</sup> McFarlane, *Biochem. J.*, 1935, **29**, 407.

<sup>8</sup> Pedersen, *J. Physic. Chem.*, 1947, **51**, 156.

<sup>9</sup> Pedersen, *Ultracentrifugal Studies on Serum and Serum Fractions* (Almquist and Wiksells, Uppsala, 1945).

<sup>10</sup> Cohn, *Experientia*, 1947, **3**, 125.

<sup>11</sup> Cohn, Strong, Hughes, Mulford, Ashworth, Melin and Taylor, *J. Amer. Chem. Soc.*, 1946, **68**, 459.



It seems probable that the various workers in this field have not attempted to account quantitatively for the lipids in plasma because of the uncertainty which surrounds their state of aggregation. Ageing accompanied by precipitation, filtration, and low-speed centrifugation individually reduce the lipid content by removing microscopically-visible particles (chylomicrons, blood platelets, etc.) which are rich in neutral fat. That sub-microscopic ones are still present in a clear filtered human serum is suggested by the presence of refraction gradients at the meniscus during preliminary acceleration of undiluted serum in the ultracentrifuge.<sup>12</sup> If the serum is covered with oil, these merge with the oil layer. Pedersen records that mixing or shaking serum with oil reduced its refractive increment, in one instance by as much as 18 %.<sup>9</sup> There can be little doubt that the systematic loss in total refraction which occurs between rest and full-speed in the ultracentrifuge is due, in some measure, to lipid micelles rising to the meniscus which are not large enough to give rise to an opalescence but are small enough to refract visible light.

The question also arises whether the same sub-microscopic particles may not give rise in the electrophoresis cell to some degree of endosmotic streaming of a kind which is visible with virus particles.<sup>13</sup> In this connection, no adequate theory has yet appeared to explain the disturbance which accompanies the descending  $\beta$ -globulin boundary in human serum. Longworth *et al.* attribute it to convection, while at the same time showing that it disappears after ether extraction of the serum.<sup>3</sup> Convection or endosmosis of lipid particles will, of course, seriously affect lipid distributions based on electrophoretic analyses, and might, for example, explain the small amounts of lipid which Blix *et al.* find in their albumin and  $\gamma$ -globulin fractions separated by electrophoresis<sup>2</sup> and which others do not confirm by alcohol fractionation.<sup>11, 14</sup>

**2. Association of Particulate Lipids with Protein.**—A visible opalescence due to chylomicrons in unfiltered human serum migrates usually with the  $\beta$ -globulin. However, an instance is recorded of it migrating mid-way between the  $\alpha$ - and  $\beta$ -boundaries<sup>15</sup> and the writer has observed it also in a lipaemic serum to migrate exactly with the  $\alpha$ -globulin. There appears to be no evidence of  $\gamma$ -globulin associating with particulate lipids or other materials in plasma, presumably because of the smaller number of charges which the molecule carries.

In general, arising out of the studies of Abramson, Moyer *et al.*,<sup>16</sup> all plasma proteins presumably are capable in some degree of stabilizing lipid particles. However, when present together the question of mutual displacement arises and it seems in general that  $\beta$ -globulin exercises the highest affinity for most forms of lipid, being in this respect analogous to the rare globulin fraction which is responsible for stabilizing the fat particles in milk.<sup>16</sup> When  $\beta$ -globulin is absent or fully used up (e.g. in a gross lipaemia)  $\alpha$ -globulin and possibly albumin take over. However, there is not sufficient data to exclude the possibility that different lipids are stabilized by different proteins.

**3. Association of "Dissolved" Lipids with Proteins.**—In

<sup>12</sup> Personal observation.

<sup>13</sup> McFarlane, *Trans. Faraday Soc.*, 1940, **36**, 257.

<sup>14</sup> Edsall, *Advances in Protein Chemistry* (Academic Press Inc., 1947), **3**, 833.

<sup>15</sup> Blix, *J. Biol. Chem.*, 1941, **137**, 495.

<sup>16</sup> Abramson, Moyer and Gorin, *Electrophoresis of Proteins* (Reinhold Publishing Corporation, 1942).

recent years most workers have come to associate the dissolved or molecularly-dispersed lipids of human plasma with the *X*-protein. Pedersen, to whom the characterization of this protein is mainly due, finds that it represents an unspecified portion of the  $\beta$ -globulin boundary and proposes for it a minimum molecular weight of 500,000 and a composition of 20 % lipids, 10 % carbohydrate with albumin and globulin in a molar ratio of 3/1.<sup>9</sup> The density of the hydrated particle from centrifugation data is 1.03 g. cm.<sup>3</sup>. In view of the relatively high densities of proteins (1.37) and carbohydrates generally (1.54) it is difficult to see how a particle of this composition could have such a low density. Indeed the value is so close to that of mixed plasma lipids<sup>17</sup> (1.004) as to suggest that this so-called "protein" must consist mainly of lipids. Petermann, using Pedersen's method, has also prepared a fraction rich in *X*-protein, and has studied the release from it of particulate lipids which is known to result from the action of lecithinase.<sup>18</sup> While she makes no estimate of density it is noticeable in her ultracentrifugal analyses that after the enzymatic "release" of 51 % *X*-protein, the albumin/globulin ratio is sensibly unchanged. This can be interpreted to mean that the *X*-protein consists almost entirely of lipids or alternatively that any protein which it contains must be representative of the plasma proteins as a whole. This would appear to be a simple matter to settle by chemical analysis of pure material, but unfortunately serious losses occur in attempts to wash the crude material.<sup>8</sup> The instability is also reflected in the case of the Harvard lipo-proteins by high osmotic pressures, suggesting the presence of smaller molecules, "very probably of lipid", and indeed leakage of lipids through the membranes is also recorded.<sup>6</sup>

Pedersen's purified *X*-protein shows two approximately equal peaks on ultracentrifugation in the presence of salt, one with the sedimentation constant of albumin. Since the proportion which this purified material bears to the *X*-protein in the original serum is not stated, it is difficult to estimate the significance of the free albumin or indeed of the purified *X*-protein. In view of the readiness with which the albumin evidently dissociates it is not surprising that Oncley *et al.* do not find any of it in their purified lipo-protein fractions. At the same time it is pertinent to ask whether such a loose association of albumin with lipids and globulin can have any important significance in the original plasma. The writer wonders whether, in estimating more albumin than globulin to be present, Pedersen may have been influenced by the high concentration (up to 28 %) which he finds,<sup>9</sup> and which Petermann confirms,<sup>18</sup> for the *X*-protein in slightly diluted human serum. It represents more than the combined concentrations of the  $\alpha_1$  and  $\beta_1$  globulins.<sup>19</sup> However, Johnston and Ogston have now shown that the concentration effect which is present in the ultracentrifuge at high total protein concentrations, and which gives rise to large apparent concentrations of albumin at the expense of the globulins, is due to a boundary anomaly<sup>20</sup> and not, as had been suggested, to a splitting of globulin molecules.<sup>7</sup> That this effect is present in Pedersen's case is seen from the fact that the concentration of the

<sup>17</sup> Popjak and McCarthy, *Biochem. J.*, 1946, **40**, 789.

<sup>18</sup> Petermann, *J. Biol. Chem.*, 1946, **162**, 37.

<sup>19</sup> Armstrong, Budka, Morrison and Hassam, *J. Amer. Chem. Soc.*, 1947, **69**, 17.

<sup>20</sup> Johnston and Ogston, *Trans. Faraday Soc.*, 1946, **42**, 789.

X-protein increases from 11 % at 3.2 % total proteins to 28 % at 5.3 % proteins. While it is not possible to say precisely what is the true proportion of X, it is definitely not more than 11 % and may be much less. The total lipids of normal human plasma are equivalent to 7 % of the proteins.<sup>21</sup>

The Adairs obtain a mean molecular weight of 350,000 for their  $\beta$ -globulin by osmotic methods and show by immunological tests that it contains neither albumin nor  $\gamma$ -globulin.<sup>5</sup> The molecular homogeneity of the X-protein is also doubtful. The asymmetric portion of the albumin curve in the ultracentrifuge diagram has been resolved graphically into two or three homogeneous components<sup>9</sup> but a larger number might be accommodated.

In proceeding to consider the nature of the association of lipids and proteins at the molecular level it appears that the main practical criterion of this is the optical transparency of the solutions, and probably the most serious single obstacle in the way of studying the association lies in our inability to produce equally clear solutions of lipids and plasma proteins *in vitro*. Some experiments in this field are reported below.

### Experimental

Efforts to disperse cholesterol, cholesterol oleate or triolein into rabbit serum resulted in the production of opaque emulsions in which only insignificant amounts of lipids went into clear solution. These experiments differ from numerous predecessors only in the use of a powerful ultrasonic generator which comprises an X-cut quartz crystal of 7 cm. diam. dissipating 10 W cm.<sup>-2</sup> into oil at 300,000 c./sec. The energy was transmitted from the oil to the serum with negligible loss through a cellophane diaphragm or alternatively, with up to 30 % loss, through the wall of a test tube. Radiation was not prolonged beyond 5 min. since protein denaturation then becomes evident. It may be noted that a few seconds' irradiation disperses completely the micellar cloudiness associated with some solutions of sodium oleate or bile salts.

Efforts were also made to obtain combination of cholesterol and plasma proteins by a common solvent technique using 60 % normal or isopropyl alcohols. Whereas Stållberg and Teorell were able in this way to obtain a sufficient degree of solution of lipids and proteins for surface studies,<sup>22</sup> the writer failed to get a final concentration of cholesterol in rabbit serum in any way comparable to what is present in normal human serum. Neither the cholesterol nor the plasma globulins are sufficiently soluble to make this a practical bulk procedure. Attention was then turned to the less hydrophobic phospholipids.

Lecithin was prepared from rabbit brain by acetone-methanol extraction followed by repeated resolution in ether and precipitation with acetone to get rid of cholesterol and neutral fat. Cephalin was separated by precipitation with ethanol at  $-35^{\circ}\text{C}$ <sup>23</sup> and the lecithin was emulsified three times with water and flocculated with minimal quantities of acetone to get rid of water-soluble impurities. The final product gave opaque emulsions with water but ultrasonic irradiation steadily reduced the micellar size until the solution was clear by transmitted light. Lecithin was also prepared from egg yolk by essentially the same procedure but all preparations made in this way differed from the brain ones in resisting

<sup>21</sup> Peters and Van Slyke, *Quantitative Clinical Chemistry, Interpretations* (Ballière, Tindall and Cox, London, 1946), 1, 469.

<sup>22</sup> Stållberg and Teorell, *Trans. Faraday Soc.*, 1939, 35, 1413.

<sup>23</sup> Sinclair, *Can. J. Res.*, 1948, 26, 777.

all efforts, including the use of ultrasonics, to obtain optically clear solutions.

Since it is notoriously difficult to prepare proteins in bulk free of traces of lipid, e.g. for X-ray analysis, it was thought that the converse might be true, and that the difference in behaviour of the two lecithins might be attributable to traces of proteins associated with them. 1 g. of egg lecithin was therefore digested with 0.05 mg. crystalline pepsin for 2 hr. at pH 3.0 and further digested with 0.05 mg. crystalline trypsin for 2 hr. at pH 8.5. It can be assumed that the trypsin digested the residual pepsin but it is uncertain that the aqueous washing procedures which followed were any more successful in removing adsorbed residual trypsin than they were in removing protein associated with the original material. That the digestion procedures were effective is shown by a fall in nitrogen of one sample of lecithin from 2.22 % to 1.85 %, while the phosphorus remained unchanged at 4.01 %. In spite of this treatment the egg lecithin preparations still could not be obtained in clear solution.

Dropwise addition of a solution of mixed bile salts produced with the help of ultrasonics a solution as clear as that of the brain preparation with ultrasonics alone, when the proportion of lecithin to bile salts was 2/1. This solution and a brain lecithin solution were separately mixed with rabbit serum in a proportion of two parts by weight of serum proteins to one of lecithin and the two dialyzed and examined by electrophoresis. In both cases the serum globulins were unaffected both in mobility and concentration by the presence of the lecithin. In one experiment the globulins were separated in the descending limb of the U-tube and shown to contain no more than their normal amount of lipid phosphorus.

In the course of dialyzing lecithin, either alone or in the presence of rabbit serum, slow leakage of lecithin but not of serum proteins was observed. This amounted to a loss of 15 % of the lecithin in the dialysis period of 2½ days prior to electrophoresis. The lecithin which passed through the cellophane was mainly polymerized and accumulated without mixing as a milky layer at the bottom of the buffer solution. Presumably lecithin molecules or small micelles which have passed through the membrane rapidly form larger micelles in the buffer.

## Discussion

The association of proteins with lipids in the visible particles of human plasma is generally accepted to involve only van der Waals' attractive forces. It has some features, however, which distinguish it from an artificial emulsion of lecithin in plasma. The particles are charged differently and in the case of the natural material they are not in equilibrium with molecular lecithin since no leakage takes place through cellophane. They also do not give rise to any measurable osmotic pressure. It would appear that the body has some mechanism which we have not been able to imitate by which it assembles lipids in proper proportions, possibly including traces of still unrecognized materials, and assembles the whole inside an  $\alpha$ - or  $\beta$ -globulin envelope which effectively isolates the micelles.

The same situation may arise with the invisible lipid particles of plasma, with the additional difficulty that the existence of a lipo-protein molecule has to be considered. The latter term may be taken to imply any of several properties of conjugated proteins, viz. electro-valency or co-ordination valency bonds between the constituents, constancy of chemical composition and physical properties, immunological specificity, and possibly crystallinity or some biological property not

possessed by either constituent alone. From the evidence reviewed here there seems to be little to justify classifying the various lipo-proteins which have been isolated as conjugated proteins. This may of course be due to technical difficulties in preparing and studying them and particularly to an instability which seems to increase as they are purified. However, the alternative possibility must be considered that the form of association of lipids and proteins at the molecular level is not fundamentally different from that in the visible region.

By analogy with soap-hydrocarbon associations we may visualize lipids either as sandwiched between laminar sub-units of globulin molecules or enclosed in a protein skin of polypeptide side-chain thickness. In either case an upper limit is placed on the size of the particle by the maximum area which a plasma globulin molecule can occupy. In close proximity with a concentrated lipid phase it may be assumed that the protein film cannot be of the expanded or gaseous type and will therefore have an area close to 0.8 sq. m./mg. irrespective of the nature of the protein.<sup>24</sup> For a single globulin molecule of weight 156,000 this corresponds to  $2 \times 10^{-16}$  m.<sup>2</sup> which is sufficient to cover a lipid sphere of radius 35 Å. If this spherical shell is packed with mixed plasma lipids of mean density 1.00 g. cm.<sup>-3</sup> the equivalent molecular weight of the lipo-protein particle is 260,000 and the lipid content 41 %. Corresponding maximum equivalent molecular weights for micelles stabilized in this way by 2, 4, 8 and 20 globulin molecules are 0.34, 1.0, 3.2 and 13 millions, and corresponding lipid contents are 52 %, 63 %, 72 % and 80 %. It is evident from these figures that a micelle containing 75 % lipids and having a coherent globulin surface must have a molecular weight much greater than the highest yet proposed (1.5 millions) for any lipo-protein of plasma. Conversely, on any principle of lipo-protein association, if the molecular weight of a particle containing 75 % lipids is less than 4.5 millions, the surface cannot be exclusively globulin in nature.

Since plasma lipids migrate with the  $\alpha$ - and  $\beta$ -globulin boundaries the tendency has been to assume that the lipid particles are coated with  $\alpha$ - or  $\beta$ -globulins and of course this offers much the simplest explanation of the detergent effect of the proteins. Nevertheless, this can only be true if the lipids and proteins associate by simple adsorption since it is unlikely that proteins could retain their electrophoretic characteristics while entering into any kind of intimate bonding with such large proportions of lipids. The identification of two components, in both  $\alpha$ - and  $\beta$ -globulins, only one of which contains lipids,<sup>6</sup> suggests the possibility that the surface properties, and hence the mobilities, of  $\alpha_1$ - and  $\beta_1$ -globulins are to some extent determined by the lipids. In such a case the possibility exists of true conjugation between lipid and protein components, and relatively small particle weights can be reconciled with high lipid contents. Decisions on these matters must clearly await more precise mobility data for isolated lipo-proteins.

The writer is indebted to Mrs. A. Dovey for making the electrophoresis observations mentioned in the experimental section.

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<sup>24</sup> Bull, *Advances in Protein Chemistry* (Academic Press Inc., 1947), **3**, 95.

# BLOOD PLASMA LIPO-PROTEINS, WITH SPECIAL REFERENCE TO FAT TRANSPORT AND METABOLISM

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Some of the more important factors affecting the blood lipids likely to be concerned in blood plasma lipo-proteins are considered. Experimental data are stabilized by various systems. The effects of a protein environment on these various fat particles are described. Various artificial emulsion are compared and contrasted with the chylomicron emulsion. The possible significance of blood lipo-proteins in relation to the transport of lipids, fat oxidation and tissue structure are discussed.

A lipo-protein is more than a simple admixture of lipid and protein ; its specific properties must be dependent upon the association of the lipid and protein molecules ; the binding forces between the lipid and protein molecules may take a variety of forms. The strength and characteristics of the bonds which hold the lipids and proteins together are important biologically in so far as they may affect biological properties. Lipo-proteins have so far been studied mainly from the physical and chemical aspect ; the relationship to biological function may perhaps be discussed most easily either in relation to cell structure or to blood plasma lipo-proteins. The object of this paper is to put forward some experimental and biological observations which may be relevant to the biological chemodynamics of blood lipo-protein complexes.

The lipids in blood have a more or less constant level which may be observed in man after about 10-12 hr. starvation. This basic level varies with age and sex, and under the influence of certain hormones. It may also vary in disease. Marked variation in lipoids is always observed after the ingestion of lipid-containing foods and after starvation for longer periods than 12 hr. In some animals marked changes in lipids may also result from repeated haemorrhage, ether anaesthesia high cholesterol diet and other causes. The commonest physiological variation is the systemic post-absorptive hyperlipaemia which follows the ingestion of a fat-containing meal. The physiological characteristics of this hyperlipaemia have been extensively studied.<sup>1, 2, 3</sup> This post-absorptive hyperlipaemia is used as a basis for the studies reported in this paper.

Normal human blood plasma contains lipids in three main forms : fatty acids and their glyceryl esters, phosphatides and sterols.

**Fatty acids and their glyceryl esters.**—If a lipaemic blood is collected after a period of 12 hr. starvation, it only contains a trace

<sup>1</sup> Gage and Fish, *Amer. J. Anat.*, 1924, **34**, 1.

<sup>2</sup> Frazer and Stewart, *J. Physiol.*, 1937, **90**, 18.

<sup>3</sup> Elkes, Frazer and Stewart, *ibid.*, 1939, **95**, 68.

of fatty acid and glyceryl esters. In normal post-absorptive hyperlipaemic blood the glyceryl esters are markedly increased, but there is no significant alteration in the quantity of free fatty acid. The esters observed in this hyperlipaemia are triglycerides, mainly containing long-chain ( $C_{12}$  to  $C_{18}$ ) fatty acids. The more unsaturated and short-chain fatty acids are not found in these triglycerides. The long-chain triglycerides occur in the blood as particles forming an oil-in-water emulsion. The sizes of the particles varies up to  $0.5 \mu$  diam., with occasional larger particles up to  $1.0 \mu$  diam.

In man the particulate fat in the blood in post-absorptive lipaemia is essentially derived from triglyceride fat in the diet.<sup>2</sup> These long-chain triglycerides are partly hydrolyzed in the intestinal lumen and emulsified to a particle size of less than  $0.5 \mu$  in the small intestine by an emulsifying system consisting of fatty acids, lower glycerides and bile salts.<sup>4</sup> This finely dispersed triglyceride passes into the intestinal cell.<sup>5</sup> Alternatively, the triglycerides are completely hydrolyzed in the intestinal lumen and re-synthesized again in the intestinal cell.<sup>6</sup> Whatever the method of transport through the outer border of the intestinal cell, it is generally agreed that particulate triglyceride accumulates within the intestinal cell and that this passes through the inner border of the cell into the corium of the villus.

If negatively charged particulate material is injected into the tissues it does not pass into blood capillaries, but is conveyed by the lymphatic vessels to the nearest lymphatic glands. Negatively charged fat particles behave similarly in the intestinal villus and pass into intestinal lymphatics and not into the portal blood vessels. When fat is being absorbed the quantity of lymph passing up the thoracic duct is greatly increased. This lymph must be derived from blood reaching the intestinal villi. There must, therefore, be an alteration in the permeability of the intestinal capillaries during fat absorption which allows the formation of much larger quantities of lymph. Little change in lymph flow occurs during the absorption of carbohydrates or amino acids. The mechanism, causing this increased lymph flow, has not yet been investigated. It seems probable that the flow of lymph, but not its formation, is assisted by movements of the intestinal villi. The lymph formed is similar to blood plasma, except that it contains less protein, usually about 4 g./100 ml. The albumin/globulin ratio may be increased.

The fat-laden lymph now passes up the thoracic duct, short-circuiting the liver, and is injected directly into the venous blood just before it enters the heart. The fat particles now circulate in the systemic blood, from which they are removed, mainly into the fat depots.

**Phosphatides.**—The main phosphatide in the blood is said to be lecithin and the normal level is about 250 mg./100 ml. Natural lecithin may not conform to the generally accepted formula for lecithin, since many of its characteristics cannot be explained on this basis. Differences are found in the fatty acids in the lecithin molecule. Some samples contain very unsaturated fatty acids, such as arachidonic acid; others contain more saturated compounds. More detailed information is required with regard to the chemistry of blood phosphatides. Con-

<sup>4</sup> Frazer, Schulman and Stewart, *J. Physiol.*, 1944, **103**, 306.

<sup>5</sup> Frazer, *Physiol. Rev.*, 1946, **26**, 103.

<sup>6</sup> Verzar and McDougall, *Absorption from the Intestine* (Longmans, Green and Co., London, 1936.)

fusion is caused by the use of the term "lecithin" for a variety of phosphatides, which differ fundamentally in their chemical and physical properties.

Phosphatides can be synthesized in the liver, small intestine, kidney,<sup>7</sup> and possibly other organs. During fat absorption phospholipid synthesis has been demonstrated both in the intestinal cell<sup>8</sup> and in the liver Chyle collected during fat absorption contains lecithin, but this is not necessarily derived from material synthesized in the intestinal cell. Phosphatides in the chyle may be derived from the blood, giving rise to the lymph, or from the liver, or from synthesis in intestinal cells. Isotope-labelled plasma phosphatides have been shown to return in the lymph.<sup>9</sup> At the height of fat absorption, the increase in the blood phospholipid must be due to synthesized phospholipid passed into the blood from or through the liver rather than in the chyle, since the concentration in the latter is usually less than that in the blood. From isotope studies in normal and hepatectomized animals, it seems clear that the liver is the main site of both formation and removal of plasma phospholipids.<sup>10</sup> In addition to changes in plasma phosphatides, alteration in the lecithin content of the red blood corpuscles during fat absorption has also been described.

**Sterols.**—The main sterol in the blood is cholesterol, which occurs in free and esterified form. The normal amount observed in the blood is 250 mg./100 ml., rather more than half being esterified. Cholesterol is probably the mother substance from which sterol hormones, bile salts and other important substances are made in the body. Cholesterol is absorbed from the intestinal tract probably in association with fats. Cholesterol can be synthesized in the body.<sup>11</sup> During fat absorption, cholesterol can be demonstrated in the chyle, but it is not known whether this material comes from the diet, the bile, or the blood.

The basic level of blood cholesterol remains fairly constant. It increases, however, with age and also in pregnancy. The blood cholesterol is also related inversely to thyroid activity. In the sprue syndrome, the blood cholesterol is usually low.<sup>12</sup> It is possible that this low blood cholesterol gives rise to secondary deficiencies of steroid hormones. The low level of blood cholesterol in the sprue syndrome may be due to faulty cholesterol synthesis as the result of modified liver function.

## Experimental

The experimental observations put forward have been made over a number of years in connection with several different biological problems. They are concerned with studies on oil-in-water emulsions designed to illustrate the structure and properties of different types of fat particles and the relationship of structure to their behaviour in a protein environment.

**Materials.**—Pure substances were used in these studies as far as possible. Natural materials were prepared by standard methods, unless otherwise stated. The protein fractions were obtained by ammonium sulphate precipitation.

<sup>7</sup> Chaikoff, *Physiol. Rev.*, 1942, **22**, 291.

<sup>8</sup> Sinclair, *J. Biol. Chem.*, 1929, **82**, 117.

<sup>9</sup> Reinhardt, Fishler and Chaikoff, *ibid.*, 1944, **152**, 79.

<sup>10</sup> Entenman, Chaikoff and Zilversmit, *ibid.*, 1946, **166**, 15.

<sup>11</sup> Channon, *Biochem. J.*, 1925, **19**, 424.

<sup>12</sup> Frazer, *Brit. Med. J.*, 1947, **2**, 641.



**Methods.**—EMULSIFICATION was carried out with the Impulsor emulsifier.<sup>13</sup>

DETERMINATION OF PARTICLE SIZE, DISPERSION AND STABILITY was carried out by examination, using dark-ground microscopy.

MOBILITY was investigated by microelectrophoresis.

FLOCCULATION REACTIONS were studied by the standardized methods, as described by Elkes, Frazer, Schulman and Stewart.<sup>14</sup>

LECITHINASE REACTION was carried out by the method described by Elkes and Frazer.<sup>15</sup>

CHEMICAL ANALYSES were carried out for fats and fatty acids by the micro technique of Schmidt Nielsen.<sup>16</sup> Standard methods were used for other analyses.

## Results

(a) **Characteristics of different types of fat particle.**—Many different varieties of oil-in-water emulsions have been studied. Olive

TABLE I.—PROPERTIES OF ARTIFICIAL EMULSIONS.

	Average particle size in $\mu$	Charge	Stability		Centrifugal packing	Lecithinase
			pH 6.0	pH 8.0		
Glyceryl monostearate . . . . .	1	Nil	Bad	Bad	—	—
Sodium oleate . . . . .	0.5	Neg.	Bad	Good	Breaks	Nil
Sodium hexadecyl SO <sub>4</sub> . . . . .	0.5	Neg.	Good	Good	Breaks	Nil
Hexadecyl ammonium bromide . . . . .	0.5	Pos.	Good	Good	Breaks	Nil
Cholesterol . . . . .	1	Nil.	Bad	Bad	—	—
Bile salts . . . . .	2	Neg.	Fair	Fair	Breaks	Nil
Egg lecithin . . . . .	0.5	Neg.	Good	Good	Breaks	Breaks
Soya bean phosphatides * . . . . .	0.5	Neg.	Good	Good	Breaks	Nil
Plasma protein . . . . .	0.5	Neg.	Good	Good	Nil	Breaks
De-fatted plasma protein * . . . . .	5	Neg.	Bad	Bad	Breaks	Nil. or slight effect
Plasma albumin . . . . .	1	Neg.	Fair	Fair	Nil	Nil or slight effect

\* Elkes (1949).

oil was the dispersed phase in each case. The main properties of some of the more interesting emulsion particles are shown in Table 1. It will be seen from this table that soaps, phosphatides, or plasma proteins were equally effective as emulsifying agents, particles of an average diameter of 0.5  $\mu$  being obtained in each case. The stability of these emulsions over a pH range varied, stability being maintained in an acid medium with hexadecyl sulphate, phosphatides and proteins. Sodium oleate emulsions were unstable in an acid medium. Packing of the particles by centrifugation caused creaming and eventual breaking of all emulsions, except those stabilized by protein. The Cl. welchii *d*-lecithinase only affected emulsions stabilized with egg lecithin or plasma proteins.

(b) **Observations on emulsion particles, using mixed systems.**—Many different combinations of these and other substances have been

<sup>13</sup> Frazer and Walsh, *J. Physiol.*, 1933, **78**, 467.

<sup>14</sup> Elkes, Frazer, Schulman and Stewart, *Proc. Roy. Soc. A.*, 1945, **184**, 102.

<sup>15</sup> Frazer, Elkes, Sammons, Govan and Cooke, *Lancet*, 1945, **1**, 457.

<sup>16</sup> Schmidt-Nielsen, *Acta. physiol. Scand. D.*, 1946, **12**, Suppl. 37.

investigated. The most interesting from the biological point of view are :

(i) **SODIUM OLEATE-PROTEIN STABILIZATION.**—As already pointed out, sodium oleate emulsions rapidly break in an acid medium. They are also rapidly destroyed by 1 % sodium chloride. If plasma protein is added to sodium oleate at pH 7.4 particles are formed which are resistant to the action of sodium chloride or acidification. The sodium oleate stabilized fat particles can be similarly protected by other proteins.<sup>17</sup> This effect of protein is probably due to the formation of an adsorbed protein layer on the surface of the particle.

(ii) **DE-FATTED PLASMA-FAT EXTRACT OR EGG LECITHIN.**—The emulsifying properties of plasma are markedly reduced by de-fatting the plasma at low temperature. If the removed fat or egg lecithin are added to the de-fatted plasma, the emulsifying properties are restored. The simple addition of the extracted fatty material does not, however, completely restore the normal emulsifying action.

(iii) **GLYCERYL MONOSTEARATE-BILE SALTS-FATTY ACIDS.**—This combination was found to produce fine emulsification over a wide pH range. It is the probable emulsifying system in the small intestinal lumen.<sup>4</sup> The fatty acid and monoglyceride are formed during the hydrolysis of triglycerides by pancreatic lipase.<sup>18</sup>

(c) **Flocculation reactions of fat particles in a protein environment.**—The behaviour of fat particles in a protein environment has been extensively studied.<sup>14</sup> It can be shown that proteins are adsorbed to the charged oil-water interface and cause a characteristic flocculation over a pH range. With negatively charged particles, flocculation occurred on the acid side of the isoelectric point of the protein and with positively charged particles on the alkaline side. Considerable overlap occurred in the case of  $\gamma$ -globulin. The maximum clarification occurred when a monolayer concentration of protein was added and the flocculated particles did not coalesce. If smaller quantities of protein were added, the flocculated emulsion particles rapidly coalesced, liberating free oil. When much larger amounts of protein were added, further secondary adsorption of protein could be demonstrated.

If negatively charged fat emulsion particles are added to plasma proteins at pH 7.4, rapid flocculation occurs. If, on the other hand, fat particles stabilized with lecithin are added to plasma proteins, they remain well dispersed. A small range of flocculation is observed with lecithin-stabilized particles in the presence of protein at pH 5.5-6.0.

(d) **Comparison of artificial emulsions and the chylomicron emulsion.**—The characteristics of the normal chylomicron emulsion as obtained in post-absorptive lipaemic human plasma are shown in Table II, compared with various artificial emulsions. It will be seen that the chylomicron particle is about  $0.5 \mu$  in diam. and negatively charged. It remains discrete and well dispersed over a pH range of 4.0-10.0. Creaming is not observed after centrifugal packing. It is not affected by 1 % sodium chloride. The particle shows active Brownian movement at pH 7.4, but the mobility of the particle is decreased as the pH is shifted towards the acid side. Over a pH range immobility and flocculation can be observed at pH 5.5-6.0 ; on either side of this region free mobility is found, but the charge on the particle is reversed on the acid side. If the chylomicron emulsion is acted upon by lecithinase, flocculation and breaking of the emulsion is observed. The characteristics of the artificial emulsion particles have already been described. It will be seen that triglyceride particles, emulsified in plasma protein, seem to be identical with chylomicrons. A lecithin-stabilized emulsion shows some similarity.

(e) **Comparison of fat particles in the chyle and the chylomicron**

<sup>17</sup> Frazer and Stewart, *J. Physiol.*, 1939, **95**, 5, 7.

<sup>18</sup> Frazer and Sammons, *Biochem. J.*, 1945, **39**, 122.

TABLE II.—COMPARISON OF CHYLOMICRONS AND ARTIFICIAL EMULSIONS.

## Artificial Emulsion Particles Stabilized with

	Chylomicron	Plasma protein	Albumin	Lecithin	Sodium oleate
Average particle size	0.5 $\mu$	0.5 $\mu$	0.5 $\mu$	0.5 $\mu$	0.5 $\mu$
Charge	Negative	Negative	Negative	Negative	Negative
pH range	Flocculation pH 5.5-6.0	Flocculation pH 5.5-6.0	Flocculation pH 4.6	Flocculation pH 5.5-6.0	Breaking on acid side of pH 7.0 Floc- culation
Effect of added plasma protein at pH 7.4	No floc- culation	No floc- culation	No floc- culation	No floc- culation	
Centrifugal packing	Stability maintained	Stability maintained	Stability maintained	Breaking	Breaking
1 % sodium chloride	No effect	No effect	No effect	No effect	Breaking
Half-saturation ammonium sulphate	Flocculation particles	Flocculation particles	No effect	—	—
Saturation ammonium sulphate	—	—	Flocculation of particles	—	—
Lecithinase	Flocculation and breaking	Flocculation and breaking	No effect (or slight reaction)	Flocculation and breaking	No effect

**emulsion**—An opportunity recently occurred of obtaining fat-laden chyle from a human subject with a thoracic duct fistula. Under normal circumstances these fat particles would have been chylomicrons a fraction of a second later. The properties of these fat particles in the chyle were found to differ fundamentally from those of chylomicrons, as shown in Table III.

TABLE III.—COMPARISON OF CHYLOMICRONS AND FAT PARTICLES FROM CHYLE.

	Chylomicrons	Fat particles from chyle
Size . . . . .	0.5 $\mu$	0.5 $\mu$
Charge . . . . .	Negative	Negative
Particles migrate with . . . . .	Globulin fraction	Albumin fraction
Centrifugal packing . . . . .	Stability maintained	Stability maintained
pH range . . . . .	Flocculation, pH 5.5-6.0	Flocculation, pH 4.6
Half-saturation ammonium sulphate . . . . .	Flocculation	No effect
Saturation ammonium sul- phate . . . . .	No effect	Flocculation
Lecithinase . . . . .	Flocculation and breaking	No effect
Lecithin . . . . .	Present	Present
Cholesterol . . . . .	Present	Present
Protein . . . . .	6.5 g./100 ml.	4.1 g./100 ml.

### Discussion

Many important biological properties of proteins may be dependent upon association of the proteins with lipids. This communication is concerned with the obverse of this—the importance of association with proteins to the biological reactions of lipids. Lipo-proteins might represent a method of transport of lipids in the blood-stream, they might modify the passage of lipids through membranes, they might be concerned with fat oxidation, or they might be an essential structural component of plasma or cells. Lipo-proteins might be important biologically from all these different aspects.

(a) **Transport of Lipids.**—It has been claimed that phosphatides are important for fat transport in the blood. The first stage of fat transport is concerned with the removal of absorbed fat from the intestinal cell to the fat depots or the liver. In the case of long-chain triglycerides, for the transport of which the phosphatide mechanism might be useful, almost all the absorbed fatty material is conveyed via the thoracic duct in the form of triglycerides, and although phosphatide is present it does not appear to be essential to the stability of the triglyceride emulsion at this stage of its journey. In studies of phosphatide turnover in the intestinal cells, phospholipid does not appear to be an obligatory intermediate in the absorption of triglyceride<sup>19</sup> and it cannot be concerned with the transport in the systemic or portal blood of any large quantity of absorbed fatty material. The next step in fat transport is from the fat depots to the liver, but the available evidence indicates that again the fat is transported as a triglyceride emulsion. There is no evidence to suggest that triglyceride is converted into phosphatide during the mobilization of fat from the fat depots, nor does it appear that the mechanism of transport of fat particles from the fat depots to the liver differs in any fundamental way from the mechanism of their transport to the fat depots from the intestine. The final step in fat transport is the alleged conveyance of fats from the liver to the body cells for combustion. It can be shown that the liver forms the phosphatides which are found in the plasma. It can also be shown that the liver is mainly responsible for the eventual removal of phosphatides from the plasma.<sup>9</sup> It must therefore be concluded that no significant quantity of fat is transported in the form of phosphatide in lipo-proteins.

The primary inclusion of sterols in lipo-proteins is possible, or they may be involved secondarily. No information appears to be available at the moment on changes in sterol distribution in relation to lipo-proteins under various experimental or pathological conditions.

The main form of lipid transport in the animal body appears to be long-chain triglyceride in particulate form. It is in this form that most absorbed fat is conveyed from the intestinal cell via the lymph to the systemic blood and fat depots. It is in this form that fat is mobilized and passes from the fat depots to the liver for metabolic use. In all these movements of particulate fat in the blood lipo-proteins appear to play an essential part. Fat particles stabilized with protein alone do not show the stability or characteristic properties of chylomicrons; fat particles stabilized with lipids also differ from the particles found in lipaemic blood. The combination of properties

<sup>19</sup> Silversmit, Entenmann and Chaikoff, *J. Biol. Chem.*, 1948, **172**, 637.

observed could be due to stabilization of the fat particles by a phosphatide-containing lipo-protein. The differences between the fat particles in chyle and those in the blood at the height of fat absorption require further study. They indicate, however, the probable competitive factors which determine the nature of the stabilizing interfacial film.

(b) **Passage of lipids through membranes.**—It might be anticipated that lipo-protein formation would modify the passage of some of its component molecules through cell membranes. What significance this may have biologically is unknown. There is evidence that fat particles with different interfacial film structure are treated differently in the body. Thus some fat particles are readily taken up into the reticulo-endothelial cells in the liver and other tissues, while others pass into the fat depot cells. The essential factors determining these differences are related to the nature of the stabilizing film on the surface of the particle.

(c) **Fat oxidation.**—It has been claimed that phosphatides, formed from triglycerides, are more oxidizable and thus phosphorylation might represent an important stage in fat oxidation. The evidence for this seems to be based mainly on the rapid oxidation which lecithin undergoes in air. It is likely, however, that the unsaturated fatty acids which oxidize under these conditions are protected from oxidation when the phosphatide forms part of a lipo-protein complex. There is no evidence of rapid oxidation of lecithin in normal plasma. Whether or not lecithin may be freely oxidized elsewhere in the body, it must be concluded that there is no evidence of any extensive oxidation of the phosphatides in plasma lipo-proteins. Association with protein may decrease oxidative changes rather than increase the liability to oxidation.

(d) **Structural function.**—Lipo-proteins may be regarded as structural components upon which many of the properties of blood plasma depend. The structure of the plasma is such that it can accommodate alteration in water or lipid content over the physiological range. It is also possible that lipo-proteins take part in cell growth and repair which might account for the slow continuous turnover of phosphatide observed in hepatectomized animals.

In conclusion it may be said that lipo-proteins clearly play a vital part in cell structure and they may be important in blood coagulation, triglyceride transport and in immunity. Furthermore, the association between lipid and proteins may so modify the properties of the components of the lipo-protein, that biological activity can be directed in a particular way.

I am greatly indebted to several colleagues with whom many of the observations in this paper have been made, especially Dr. J. Elkes and Dr. H. G. Sammons. I am grateful to the Sir Halley Stewart Trust and Medical Research Council for financial assistance.

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## GENERAL DISCUSSION \*

**Prof. A. C. Frazer** (*Birmingham*) said: The association of carotenoids and oestrogens with  $\beta$ -lipo-proteins is a point of considerable biological interest. It is known that vitamin A may be associated with the glyceride fraction of blood lipids or with some other lipid fraction according to the form in which it is administered. It certainly behaves very differently from carotene. Is there any information with regard to vitamin A distribution in these lipo-proteins?

**Prof. E. C. Wassink** (*Wageningen*) said: I would like to ask Dr. Edsall whether the large numbers of (mol. lipid)/(mol. protein) in  $\beta$ -lipo-protein mean that there are a few hundred molecules of cholesterol bound (in some way) to one molecule of protein?

**Prof. E. J. Cohn, Dr. J. T. Edsall, Dr. F. R. N. Gurd and Dr. J. L. Oncley** (*Harvard*) (*communicated*): In reply to Prof. Frazer, studies of the distribution of vitamin A and vitamin E were attempted some time ago by colleagues of Dr. Hickman at Distillation Products, Rochester, N.Y. The plasma fractions then available had undergone considerable oxidative changes, and no active vitamins were found. Studies have not yet been made with lipo-proteins prepared from fresh blood under conditions that carefully protected them from oxidation.

In reply to Dr. Wassink: Yes, our studies indicate that something like a thousand moles of cholesterol (about one-quarter as free alcohol and three-quarters as fatty acid esters) are associated in some way with one molecule of  $\beta$ -lipo-protein.

**Dr. J. Elkes** (*Birmingham*) said: The absence of the  $\beta$ -lipo-protein from other than human plasma may not be unrelated to the well-nigh specific susceptibility of human serum to *Cl. welchii* lecithinase. This specificity was originally shown by Nagler<sup>1</sup> and our own observations are in keeping with his findings. Thus human and avian serum are highly susceptible to this enzyme, whereas those of horse, guinea pig and rabbit are not; though in certain instances (for example, in lipaemia of haemorrhage of the rabbit) a previously non-reacting serum may acquire a secondary susceptibility depending, presumably, upon some changes in lipo-protein constitution. It would be interesting to enquire into any differential susceptibility of the various protein fractions to this and similar enzymes, and to do so in the normal, and in lipaemias brought about by various exogenous and endogenous means.

**Dr. A. B. L. Beznak** (*Birmingham*) said: The process of ageing of the lipo-proteins in solution is probably a very complex one in which changes both in the lipid as well as in the protein moiety—followed by interactions between the two kinds of altered substances—play a role.

The first kind of process is exemplified by the work of Annau and co-workers\* showing that choline catalyzes the respiration of the lipo-proteins of *Machebocuf*. Fatty acids are believed to be burned as deduced from the *RQ* values.

In connection with the question whether the oxidation of the fixed —SH of the protein is one of the processes causing the ageing or vice versa, i.e. the ageing takes place with the appearance of fixed —SH groups followed by their subsequent oxidation, some unpublished observations—made in 1926 in the London University College—are recalled. It was found that egg-white solutions shaken in air or irradiated by ultra-violet light absorb  $O_2$  at a constant rate in direct proportion to the shaking or to the irradiation. These treatments cause turbidity and a slight flocculation also proportional to the treatment. The same relationships between shaking, ultra-violet irradiation, flocculation and formation of —SH groups are found in  $N_2$  atmosphere. Egg-white

\* On three preceding papers.

<sup>1</sup> *Brit. J. Expt. Path.*, 1939, **20**, 473; *J. Path. Bact.*, 1941, **52**, 105.

<sup>2</sup> *Hoppe-Seyler's Zts.*, 1947, **282**, 69.

solutions thus treated will take up  $O_2$ . Accordingly ageing with the formation of  $-SH$  (catalyzed by shaking or ultra-violet irradiation) takes place first and is followed by the oxidation of the  $-SH$  group previously formed.

The presence of free choline may influence, therefore, the result of the preparation of the lipo-proteins. Have Dr. Edsall and Cohn evidence of the presence of choline in their preparations?

**Dr. G. Popjak** (*London*) said: Oncley, Scatchard and Brown<sup>3</sup> observed that the mol. weight of their  $\alpha_1$  and  $\beta_1$  lipo-protein preparation as determined by the ultracentrifuge was much higher than by osmotic pressure measurements. They suggested that this anomaly was due to osmotic pressure exerted by the small molecular weight lipids. McCarthy and I have shown that the lipids do not exert an osmotic pressure and we think that there is another explanation for the anomalous results obtained by

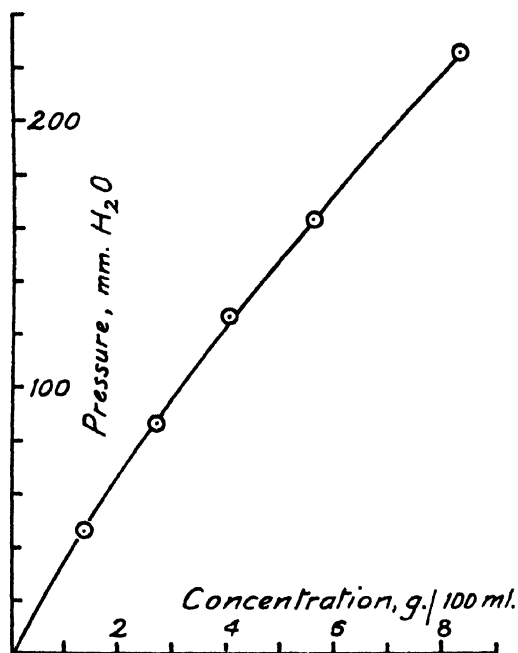


FIG. 1.—Osmotic pressure-concentration curve for  $\alpha_1$ -lipo-protein (182, N-1, 1) of Oncley *et al.*

osmometry. The osmotic pressure data of Oncley *et al.* have been plotted as shown in Fig. 1 and 2. These data are quite unusual for a protein. Adair and Adair have deduced for osmotic pressure of protein solutions at finite protein concentrations the equation

$$p = \frac{\pi_0 C}{1 - K_b C}$$

where  $\pi_0$  is the extrapolated value of the ratio  $p/C$  to infinite dilution of protein and  $K_b$  a factor equivalent to  $b$  in van der Waals' equation.  $K_b$  is equal to the product of  $\pi_0$  and the slope of the line shown in Fig. 2.

$K_b$  for the  $\alpha_1$  lipo-protein has a large negative value which is quite unusual. It is suggested that the explanation of this abnormality is not that the lipids exert an osmotic pressure, but that the protein of the

<sup>3</sup> *J. Physiol. Chem.*, 1947, **51**, 184.

$\alpha_1$  lipo-protein dissociates into smaller fragments like foetal haemoglobin which also gives a negative  $K_b$  as observed by McCarthy and Popjak.

**Prof. E. J. Cohn *et al.* (Harvard) (communicated)**: We have, of course, noted the unusual characteristics of the  $\alpha$ - and  $\beta$ -lipo-protein osmotic pressure studies as outlined by Dr. Popjak. The suggested explanation of a dissociation into smaller fragments like foetal haemoglobin was considered, but there was no evidence for such a dissociation in ultracentrifugal studies made concurrently. The observation of considerable leakage through collodion membranes impermeable to other serum protein molecules would also be difficult to explain. The suggested dissociation of lipid molecules from the lipo-protein appears to explain both this leakage and the slope of the  $P/C$  against  $C$  osmotic pressure curves.

The extent of this dissociation is, however, very small. Something of the order of 0.2 % of the total lipid from the  $\beta$ -lipo-protein, and of 1 % of that of the  $\alpha$ -lipo-protein, would be sufficient to explain the observed

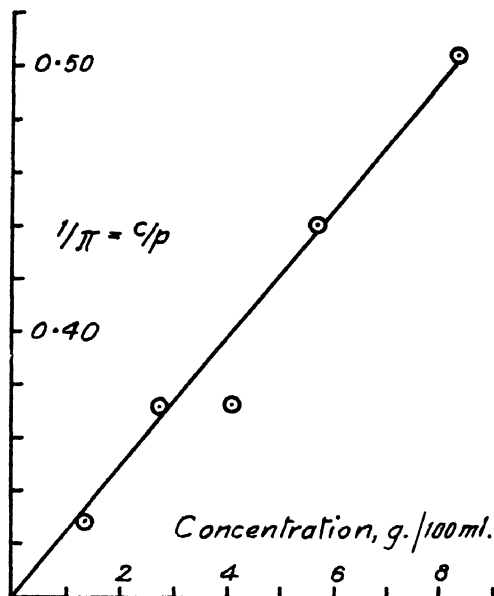


FIG. 2.—Determination of  $1/\pi_0$  by extrapolation for  $\alpha_1$  lipo-protein of Oncley *et al.* To obtain  $1/\pi$  the osmotic pressures shown in Fig. 1 have been converted to mm. Hg.

discrepancy in osmotic pressures. Thus more than 99 % of the lipid is very strongly bound to the  $\alpha$ - and  $\beta$ -lipo-proteins. Lipid binding by proteins of this kind appear to be required in order to explain the osmotic pressure effects reported by Popjak and McCarthy in their paper.

**Prof. J. R. Marrack (London)** said: The studies of lipo-proteins in human serum have a bearing on a problem of chemical pathology. In nephrosis the concentration of lipids in the serum is very much increased. These lipids are associated with  $\alpha$ - and  $\beta$ -globulin. If these lipids form complexes with special proteins, as indicated by Prof. Macheboeuf's paper, these special proteins also must be increased in nephrosis. It is possible that this increase of lipo-protein should be regarded as primarily an abnormality of protein metabolism, rather than an abnormality of lipid metabolism.

**Dr. O. Hoffmann-Ostenhof (Vienna)** said: I want to report some experiments which may have some bearing on the question of Prof. Luck's paper on the ageing of proteins in serum and organ extracts. Working



with protein solutions from various sources, we found that during the first 2 days aerobic and strictly anaerobic conditions produced exactly the same phenomena of ageing (rise in viscosity, flocculation, etc.). Thus, it can be concluded that the SH—groups are probably not involved in the ageing process. On longer standing some differences between the anaerobic and the aerobic experiments were observed, but the results proved not to be reproducible.

**Prof. E. J. Cohn** *et al.* (*Harvard*) (*communicated*): The changes which we have observed during ageing of solutions of human serum  $\beta$ -lipo-proteins have seemed to involve mainly the lipid moiety. The changes in the lipids have appeared to be due to autoxidation, and have been evident on the one hand in the gradual bleaching of the visible carotenoid spectrum and on the other hand in enhancements of absorption in the ultra-violet. Gradual increases in the electrophoretic mobility of the lipo-proteins have also been observed. The changes detected in the ultra-violet absorption spectrum are progressive, and are similar to those found during autoxidation of unsaturated fatty acids and their esters. These have been ascribed to the development of conjugated unsaturated ketones following initial formation of hydroperoxides. Such changes appear to occur in otherwise intact phospholipids and possibly also in the cholesterol fraction of the lipids. They have been somewhat retarded by storage in the insoluble state or under nitrogen, but we do not expect to be successful in completely avoiding them until contact with air is rigorously excluded at all times in preparative methods. Unfortunately, no analysis for free choline in these preparations has as yet been made.

With  $\alpha$ -lipo-proteins, very definite solubility changes have been observed on ageing, resulting in far lower solubility. Thus the  $\alpha$ -lipo-protein prepared from the blood which has been collected 72 hr. previously—as happened during World War II with blood collected by the American Red Cross—was but slightly soluble in 0.066 mole fraction ethanol at pH 5.8 and  $-5^{\circ}$ , whereas all the  $\alpha$ -lipo-protein in freshly collected blood is soluble under these conditions.<sup>4</sup>

**Prof. A. Frey-Wyssling** (*Zurich*) said: It is amazing that two mammals (horse and man) have such different lipo-proteins in their sera, as indicated by the papers by Chargaff and Edsall. Would it be possible to get a better agreement if both sera were investigated simultaneously by the same team, in the same laboratory, and by the same methods?

**Prof. M. Macheboeuf** (*Paris*) said: I have not as yet studied human serum sufficiently to be able to answer Prof. Frey-Wyssling's question. But I have frequently compared horse serum and dog serum, using precisely the same method. The difference between the two sera is considerable. The same method which gives the C.A. for horse serum leads to no comparable fraction for dog serum: nothing more is precipitated after the third precipitation at pH 4.

It is this fact, observed at the beginning of my work (1929) which induced me to concentrate my efforts on horse serum. This took place at a time when biochemists were not ready to accept any association between lipids and proteins. I wanted to demonstrate this association at least in one favourable case, horse serum. I did not even hope to isolate an intact lipo-protein, that is to say identical with one of those which I suspected to be present in serum. I wanted to obtain a lipo-protein even if this should turn out to be an artefact. This would suffice to demonstrate the possibility of linkages between proteins and lipids. In the last few years the very interesting work of the members present at this Discussion has broadened the problem; lipo-proteins have now

<sup>4</sup> Bergström and Holman, *Adv. Enzym.*, 1948, 8, 425.

<sup>5</sup> Cohn, Gurf, Gillespie, Mittelman, Derouaux, Mouton, Liu, Uroma, Lever, Kahnt, Barnes, Brown, Schmid and Surgenor, *J. Amer. Chem. Soc.* (in press).

been detected in many biological materials. The time is now ripe to make a comparative study of various animal species.

**Prof. A. C. Frazer** (*Birmingham*) said: The existence of fundamental difference between lipo-proteins in the sera of different species is also indicated by our experiments already referred to by Dr. Elkes on the effect of *Cl. welchii lecithinase* in guinea pig, rabbit and human lipaemic and alipaemic sera. The characteristic Nagler reaction in alipaemic sera is only observed in man and the flocculation of chylomicron does not occur in the guinea pig, and is seen only with certain types of lipaemia in rabbits, but appears to be a constant finding in man.

**Prof. E. J. Cohn** *et al.* (*Harvard*) (*communicated*): Petermann<sup>6</sup> has shown that  $\beta$ -lipo-protein reacts with *Cl. welchii lecithinase*. The result is drastic damage to the lipo-protein molecule. Perhaps this is related to the part of the phospholipid which we suppose to be at the surface of the lipo-protein molecule.<sup>7</sup>

Gofman, Lindgren and Elliott<sup>8</sup> have recently published a method of estimating the concentration of the "low density"  $\beta$ -lipo-protein of "X-protein," from ultracentrifugal studies of serum in a high density solvent. It is interesting that these authors state that such studies "have demonstrated the presence of such a component in about 60 % of rabbit sera."

**Prof. J. Murray Luck** (*Stanford, U.S.A.*) said: If the lipid core is large enough, such that many protein molecules would be required to form the enveloping layer, even spherical molecules might be considered capable of associating with the lipid to build up the protein envelope. But if we move in the other direction and consider lipid cores of decreasing size, we must surely reach the limit of possible envelope formation before we come to the 1/1 ratio of protein to lipid. Even our mathematical friends would find it difficult to wrap a prolate ellipsoid around a sphere. Do we not, therefore, have to discard the models containing very few molecules of protein? Otherwise we have to assume that the enveloping protein opens out and is thus denatured as it wraps itself around the lipid, or that the prolate ellipsoid undergoes a curious sort of deformation, almost an invagination, which is rather difficult to accept.

**Dr. J. T. Edsall** (*Harvard*) said: I do not know whether we should call lipo-proteins conjugated proteins. We do, I think, know at least two things about the  $\alpha$ - and  $\beta$ -lipo-proteins of human plasma: (i) there are lipid-protein complexes which move with fairly uniform velocity in sedimentation, diffusion, and electrophoresis; (ii) these preparations can be repeatedly redissolved and reprecipitated by suitable variation in pH, ionic strength, ethanol concentration and temperature, without significant change in chemical composition or physical properties. Whether true covalent bonds between the lipid and the protein moiety are involved I do not know; I do not think that any present evidence requires us to assume such bonds. Whether, in view of these circumstances, these substances should be called conjugated proteins is a matter of terminology which I shall gladly leave to others.

I must say that the molecular model suggested by Dr. McFarlane strikes me as quite arbitrary. Why, for instance, is the molecular weight of the protein component taken as 156,000? This is the molecular weight of human serum  $\gamma$ -globulin, as reported by Oncley, Scatchard and Brown; but there is no reason to assume that this protein portion of the  $\beta$ -lipo-protein has any resemblance to  $\gamma$ -globulin.

A body of experimental facts exists, which points to a molecular weight of the order of 1,300,000 for the  $\beta$ -lipo-protein. If the model leads to conclusions incompatible with these data, I should prefer to stand by the experimental findings and to throw the model overboard.

<sup>6</sup> *J. Biol. Chem.*, 1946, **162**, 37.

<sup>7</sup> Oncley, Gurd and Melin, *J. Amer. Chem. Soc.* (in press).

<sup>8</sup> *J. Biol. Chem.*, 1949, **179**, 973.

**Dr. A. S. McFarlane** (*London*) said : In reply to Dr. Edsall, I would like to point out that one implication of valency or co-valency bonding in a truly conjugated protein is that the substance may bear some specific biological property not shown by either of the constituents alone, as for example, the respiratory function in haemoglobin. If the  $\alpha$ - and  $\beta$ -lipo-proteins of the Harvard workers are conjugated in this sense, the biologist may proceed hopefully with the search for specific functions for them. If the lipid-protein association is merely a loose physical one, I would be inclined to take the view that these substances serve only a function in relation to the metabolic transport of lipids.

**Dr. J. T. Edsall** (*Harvard*) (*communicated*) : On my return to the United States I found that Dr. Oncley and Dr. Gurd had evolved a picture of the  $\beta$ -lipo-protein molecule which has certain points of resemblance to that which Dr. McFarlane has suggested here. Since the solubility properties of this substance are certainly like those of a protein and not at all like those of a typical lipid, Oncley and Gurd favour a model with as much as possible of the protein structure on the outside in contact with the solvent, and most of the lipid on the inside. However, they recognized the same difficulty Dr. McFarlane has pointed out : namely, that there are simply not enough amino acid residues in the molecule to cover the surface adequately, given the dimensions indicated by the sedimentation and viscosity measurements. They suggest that the rest of the surface of the molecule may consist primarily of phospholipids with their polar groups presumably pointing outward toward the solvent. These ideas are more fully developed in a paper by J. L. Oncley, F. R. N. Gurd and M. Melin.\*

**Prof. F. Haurowitz** (*Bloomington, Indiana*) said : The view that phospholipids are not turned over more rapidly than triglycerides is supported by our observations<sup>10</sup> on the haemin-catalyzed oxidation of linseed oil by molecular oxygen. This oxidation takes place rapidly in heterogeneous emulsions of the oil in water, but is inhibited completely by bile acids, or by alkali or organic solvents which bring about homogenization of the emulsion.

**Prof. E. C. Wassink** (*Wageningen*) said : I would like to refer to some botanical evidence which appears in accordance with Prof. Frazer's suggestion as to the inhibition of oxidation of lipids in connection with proteins. In certain photosynthetic bacteria a complex of bacterio-chlorophyll with protein exists. The bacterio-chlorophyll can be extracted with organic solvents and in this state it is so susceptible to conversions of obviously oxidative nature, that it can only be handled properly in the presence of strong reducing agents, such as, for example,  $H_2S$ . On the other hand, the pigment-protein complex can be obtained in aqueous solution, e.g. by grinding the bacterial cells. In this case the pigment proves to be quite stable under normal air conditions.

**Dr. O. Hoffmann-Ostenhof** (*Vienna*) said : With reference to Prof. Frazer's remarks on the nature of lecithin, I consider that any compound should be called a lecithin which agrees in composition and structure with that limited by the definition of a lecithin, i.e. a compound in which two OH groups of the glyceride are esterified with fatty acids of any kind and the third combined with a phosphorylated choline. In biological media there is certainly no unique particular lecithin, but on the whole the mixture is always of a fairly constant composition, when allowances are made for different organs and different species.

I would like also to ask Prof. Frazer whether there is any evidence to show that oxidation occurs on fats and lipids as such without previous hydrolysis. The existing gap in our knowledge of the processes taking place in the liver could be filled, perhaps, by investigating the possibility of the existence of a fat phosphorylating mechanism in this organ.

\* *J. Amer. Chem. Soc.*, January, 1950 (to be published).

<sup>10</sup> With Schwerin, *Enzymologia*, 1940/41.

**Prof. A. C. Frazer** (*Birmingham*) said: I would agree with Dr. Hoffmann-Osterhof's definition of lecithins, but my point was that the presence of different fatty acids might profoundly modify the biological activity of the compound. Dr. Hoffmann-Ostenhof's statement that in biological media there is certainly no unique particular lecithin is not quite true. The classical experiments of R. G. Sinclair and of Artom and his colleagues showed the incorporation of ingested fatty acids into lecithin in the intestinal cells. Clearly the composition of lecithin synthesized in the intestine may be influenced by variations in dietary fat. Lecithin from the liver, on the other hand, seems to have more generalized characteristics, particularly the inclusion of long chain ( $C_{20}$  or longer) unsaturated fatty acids. It is possible, therefore, that lecithins formed in the intestinal cells and those formed in the liver may serve different functions in the body.

With regard to oxidation without previous hydrolysis, I do not know of any positive evidence of this. It is strange, however, that while the lipolytic esterases of the liver are much more effective against short-chain than long-chain glycerides, the material mobilized from the fat depots to the liver consists essentially of long-chain glycerides. Studies in fat oxidation have been carried out on fatty acids and the question of the hydrolysis of the long-chain triglycerides to fatty acid as a preliminary to oxidation has been largely neglected.

**Dr. A. Lasnitzki** (*Birmingham*) said: May I ask Prof. Frazer whether chylomicrons resembling those observed in the systemic blood are known to occur in the interstitial space between the aggregation of fat cells constituting a fat depot and the supplying blood capillaries, and whether there is any evidence that the small lymphatics and/or veins which drain the area of a fat depot may contain chylomicrons of a similar type, particularly during fasting. It is obvious that the detection of particulate fat in these situations would give considerable support to the theory that fat is received by the fat depots, and mobilized from them, essentially in the same form in which it is present in lymph and blood.

**Prof. A. C. Frazer** (*Birmingham*) said: Dr. Lasnitzki's question raises an interesting point. It is, generally speaking, true to say that negatively charged particulate material tends to pass into lymphatics rather than into blood vessels anywhere in the body. Thus, the injection of particulate dyes into the tissues is used as a means of demonstrating lymphatics. The intestinal villus is no exception to this general rule and particulate material passes mainly into the lacteals, while water-soluble material tends to pass up the portal vein. However, particulate fat appears to pass out of blood vessels into macrophages. Fat is mobilized from the fat depots probably in particulate form and one would expect, therefore, that it would pass into the lymphatics draining the fat depot rather than into the capillaries and veins. Mobilized fat would thus enter the blood stream in the same way as absorbed particulate fat, as an "intravenous injection" via the thoracic duct. We have made some observations on lipaemia in starvation which are in accord with this conception, but systematic investigation of the whole problem is necessary before any conclusions can be reached. Of course, only a part of the fat in the fat depots is derived from blood fat—a considerable amount may be formed from carbohydrate.

**Prof. A. B. L. Beznak** (*Birmingham*) said: Regarding the mode of the passage of the chylomicrons from the blood into the parenchymal cells, histological evidence indicates as first step the activity of the fixed phagocytes (Kupfer cells) in the capillary endothelium.

The lungs are known to be the first to store the chylomicrons. Dietary fat is very quickly deposited also in the perirenal and mesenteric fat depots, as has been shown by Beznak and Hasch.<sup>11</sup>

<sup>11</sup> *Quart. J. Expt. Physiol.*, 1937, **27**, 1.

A simple class-room experiment indicates the structure of the chylomicron. The fat obtained from the lymph is pressed out of a capillary tube dipped into the lymph previously defatted by centrifugation. Each droplet will be coated by a layer of protein and rises to the surface. If ether is poured on the surface it dissolves the fat. The coating is precipitated on the lymph-ether boundary surface and consists of proteins and Ca soap.

**Dr. J. Elkes** (*Birmingham*) said: A possible means of studying fat transport and deposition may be provided by the use of finely divided artificial emulsions. These can be prepared *in vitro* relatively easily, and a particularly helpful feature is that by choice of suitable materials the "core" and the stabilizing film of the emulsion particle can be varied independently. The role played by each in determining the ultimate fate of the particle could thus conceivably be studied. In connection with work on the possible use of fat in intravenous alimentation we have recently prepared some 70 such emulsions. Plasma protein and plasma protein fractions, despeciated bovine serum and a carbohydrate, dextran, were used as stabilizers. Animal and plant phosphatides and cholesterol provided other variables. An interesting feature was the emulsifying power of human freeze-dried whole plasma, unaided by any other component. Emulsions of particle size  $\frac{1}{2}$  to  $1\ \mu$  were obtained. In terms of flocculation pattern and electrophoretic mobility over a pH range these emulsions closely resembled the natural chylomicron, and provided the protein was used in concentrations not exceeding those necessary to give a coherent monolayer around each particle, these emulsions were found to be heat-stable. When injected intravenously in doses of 1 ml. per 100 g. body weight into rats they disappeared from the circulation within 30 to 40 min. and unlike the findings with incompatible emulsions (i.e. of the type flocculated by serum proteins), no fat embolism was seen. Emulsions prepared with purified brain or cord phosphatides were tolerated equally well in acute experiment. These studies are being continued, and it is hoped that isotope techniques may prove particularly useful here.

**Prof. A. C. Frazer** (*Birmingham*) said: The importance of the lungs in relation to fat metabolism and fat deposition is difficult to assess. As Prof. Beznak says, there is an accumulation of fat in the lungs following fat absorption. We examined the lungs of human subjects who were killed during the war some hours after an evening meal—the amount of fat in the lungs was usually 10-20 % of the dry weight, whereas the control lungs contained only 1-2 %. This, however, may only be a temporary mechanical effect. The rate of removal of fat from the circulation is very rapid and marked differences in particulate fat content have been demonstrated by us between arterial, capillary and venous blood collected simultaneously from the same area.

I am inclined to doubt whether the simple class experiment described by Prof. Beznak really indicates the structure of the chylomicron. Particulate fat is protein stabilized in the chyle and in the blood, but the particles behave in a fundamentally different way in electrophoresis, protein precipitation, or in the presence of lecithinase. Artificial emulsions consisting of particles stabilized with proteins and soaps behave very differently from chylomicrons when they are introduced into the blood stream.

**Mr. W. D. Brown** (*Birmingham*) said: Some observations of Hahn suggest that heparin can cause in a matter of minutes a marked decrease in the amount of particulate fat in lipaemic blood. In addition, Weld claims that this effect is not associated with a change in total blood fatty acid content. Data of my own confirms this, although as yet these effects have not been obtained *in vitro*. In view of the very rapid decrease in particle size after *in vivo* heparin treatment, is the conventional view of the chylomicron as a globule of neutral fat surrounded

by a thin film of protein adequate? Is it possible to conceive of the particle as a more complex protein lipid aggregate?

Dr. H. L. Boolf (*Leiden*) said: Bungenberg de Jong has shown that if you mix lecithin and a sulphate colloid (e.g. carrageen) no interaction takes place, except in the presence of cations, e.g.  $\text{CaCl}_2$  produces a strong flocculation in the concentration range 0.005-0.5 mole/l. If we now assume that the chylomicron is a labile structure of fat, phospholipid and protein, it might be possible that heparin (through its sulphate groups) combines with the phosphatide in the presence of  $\text{Ca}^{++}$  ions, resulting in a disruption of the structure. The discrepancy between the experiments *in vivo* and *in vitro* might be due to the fact that the latter experiments were carried out in the absence of Ca salts.

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## THE POSSIBLE INFLUENCE OF LIPIDS ON THE OSMOTIC PRESSURE OF SERUM PROTEINS

BY G. PÓPJÁK AND E. F. MCCARTHY

*Received 20th May, 1949*

Osmotic pressure measurements were carried out on (a) normal, (b) lipaemic rabbit sera, before and after extraction of lipids, (c) normal human serum, and (d) lipaemic sera of patients with subacute nephritis of the nephrotic type. It is shown that the lipids in sera do not exert detectable osmotic pressure. The volume occupied by the lipids in the serum, however, reduces the volume of solvent available to the proteins and thus increases the osmotically-effective concentration of the latter. Therefore it is impossible to assess correctly the colloid osmotic pressure of lipaemic sera unless the protein concentrations are expressed as g. per 100 ml. of solvent.

The specific volume of unfractionated rabbit and human serum proteins (without water of hydration) was found to be 0.729 and 0.731 respectively, and that of serum lipids 0.996  $\text{cm}^3 \text{g}^{-1}$ .

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Some investigators (Macheboeuf,<sup>1</sup> Macheboeuf and Sandor,<sup>2</sup> Fishberg,<sup>3</sup> Rabinowitch<sup>4</sup>) have claimed that serum lipids exert an osmotic pressure. In our first investigation<sup>5</sup> we found that the lipids in normal human serum do not exert an osmotic pressure. In view, however, of Macheboeuf's report that lipo-protein complexes prepared from lipaemic sera of human patients suffering from lipoid nephrosis (subacute nephritis of the nephrotic type) show considerably higher osmotic pressures per gram protein than the albumin of the same sera, we decided to examine human pathological and experimentally induced lipaemic sera. The results of our experiments have been reported in detail.<sup>6</sup>

We have shown that the lipids themselves do not exert an osmotic pressure. The volume occupied by the lipids in the serum, however, reduces the volume of solvent available to the proteins and thus increases the effective concentration of the latter. Hence if the osmotic

<sup>1</sup> Macheboeuf, *Bull. Soc. Chim. biol.*, 1929, **11**, 485.

<sup>2</sup> Macheboeuf and Sandor, *ibid.*, 1931, **13**, 745.

<sup>3</sup> Fishberg, *J. Biol. Chem.*, 1929, **81**, 205.

<sup>4</sup> Rabinowitch, *Arch. intern. Med.*, 1930, **46**, 752.

<sup>5</sup> Popják and McCarthy, *Biochem. J.*, 1943, **37**, 702.

<sup>6</sup> *Biochem. J.*, 1946, **40**, 789.

pressure of a lipaemic serum is compared, at finite protein concentrations, with the osmotic pressure of a non-lipaemic serum with the same protein composition, i.e. with the same protein concentration per 100 ml. of serum and the same albumin-globulin ratio, the lipaemic serum would exert a higher osmotic pressure, merely because the effective concentration of protein is increased. Therefore it is impossible to assess correctly the colloid osmotic pressure of lipaemic sera unless the protein concentrations are expressed as g. per 100 ml. of solvent.

Our conclusions are based on the study of (a) normal, (b) lipaemic rabbit sera, before and after extraction of lipids, (c) normal human serum, and (d) lipaemic sera of patients with subacute nephritis of the nephrotic type.

### Experimental

**Production of Lipaemia in Rabbits.**—This was done by feeding amorphous cholesterol (1 g. per day) without fat in a watery suspension. It was shown (Popják<sup>7</sup>) that this method produces a marked increase in all the lipid fractions of the blood plasma, the lipaemia often reaching a level of 2,000-3,000 mg. per 100 ml. of plasma. The lipaemic sera, when in sufficient quantity, were investigated individually; otherwise the sera of two or more rabbits were pooled.

**Human Serum.**—Normal human serum was obtained from 10 healthy male medical students, equal volumes from each being pooled. Human lipaemic sera were obtained from three patients who suffered—according to all clinical and laboratory diagnostic measures—from subacute nephritis of the nephrotic type.

**Osmotic Pressure Measurements** were made at 0° by the method of Adair<sup>8</sup> using M/15 phosphate buffer (pH 6.8) and collodion sacs of such permeability that at a pressure of 480 mm. Hg the rate of filtration of water through the membranes was 0.0015-0.0030 ml. cm.<sup>-2</sup> min.<sup>-1</sup>. Membranes with 30-35 cm.<sup>2</sup> total surface area were used. The osmotic pressures were measured in mm. of the protein solutions and were calculated as mm. Hg after determination of the densities of the solutions by pycnometry at 0°. Corrections for capillarity forces were made.

**Protein Concentration** was measured by determination of nitrogen by a micro-Kjeldahl method. For the determination of albumin-globulin ratios the globulins were precipitated by saturation with MgSO<sub>4</sub> and the albumin-N determined on the clear filtrate.

Although the MgSO<sub>4</sub> method is tedious, we have shown<sup>9</sup> by electrophoretic examination of the MgSO<sub>4</sub> filtrate and of the precipitated globulins that this method achieves a practically clean-cut separation of serum proteins into albumin and globulins, both in normal and lipaemic sera.

Total protein concentration C in g. per 100 ml. solution was obtained by the formula

$$C = \left( 6.41 + 0.2 \times \frac{\text{g. of globulin-N}}{\text{gl of total N}} \right) \times \text{g. of total protein-N,}$$

assuming 15.6 % N content for albumin and 15.13 % for globulins (Adair and Robinson<sup>10</sup>). Corrections for phospholipid-N were made, 1.8 % being taken as the mean N content of phospholipids.

**Lipid Analyses** were carried out as described previously (Popják<sup>7</sup>). The lipid contents of the sera are shown in the Tables as mg. per g. total protein; this method of presentation was chosen instead of the customary g. per 100 ml. because it allows the calculation of the lipid content, from the protein concentration, of all the serum-dilutions used in the osmometry.

<sup>7</sup> Popják, *Biochem. J.*, 1946, **40**, 608.

<sup>8</sup> Adair, *Proc. Roy. Soc. A*, 1925, **108**, 627.

<sup>9</sup> Popják and McCarthy, *Biochem. J.*, 1946, **40**, 789.

<sup>10</sup> Adair and Robinson, *ibid.*, 1930, **24**, 993.

## Results

Table I shows the lipid content of the normal and of the lipaemic rabbit sera used for osmotic pressure measurement. It can be seen that

TABLE I—LIPID CONTENT OF NORMAL RABBIT SERUM AND LIPAEMIC RABBIT SERA, NO. 1 AND 2 USED FOR MEASUREMENT OF OSMOTIC PRESSURES

	Normal Serum <sup>1</sup>	Lipaemic Serum No. 1 <sup>2</sup>	Lipaemic Serum No. 2 <sup>3</sup>
	(Mg./g. Total Serum Protein)		
Neutral fat . . . . .	8.5	43.5	119.0
Phospholipids . . . . .	12.9	65.0	78.5
Cholesterol { free . . . . .	2.4	46.0	58.5
ester . . . . .	8.9	118.0	129.0
Fatty acids of cholesteryl esters .	6.4	85.0	93.0
Total lipid . . . . .	39.0	346.0	479.0

<sup>1</sup> Pooled serum of three normal rabbits.

<sup>2</sup> Pooled serum of two cholesterol-fed rabbits (Ch. 5 and 6).

<sup>3</sup> Serum of cholesterol-fed rabbit (Ch. 3).

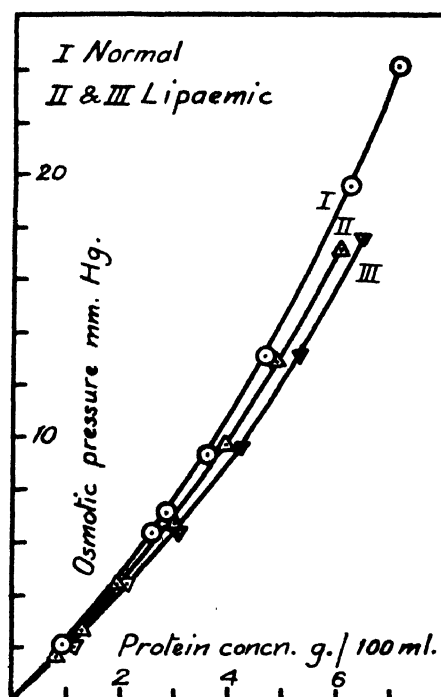


FIG. 1.—Relationship between protein concentration and osmotic pressure of (I) normal, (II) and (III) lipaemic rabbit sera No. 1 and 2 respectively. The continuous curves have been calculated by the equation  $p = \Pi_0 C / (1 - a \Pi_0 C)$ , where  $a$  = the slope of the corresponding straight lines in Fig. 2.

○ Observed values for normal; △ and ▽ for lipaemic rabbit sera No. 1 and 2

cholesterol-feeding produced great increase in all the lipid fractions of the serum and it would seem, therefore, that if any of the serum lipids



exert an osmotic pressure or influence the colloid osmotic properties of the proteins, such effects should be detectable with these sera.

In Fig. 1 the osmotic pressures of the 3 sera are plotted against protein concentration, from which it can be seen that the lipaemic sera (curves II and III) exerted a lower osmotic pressure per g. of protein than did the normal serum (curve I).

The observed osmotic pressure  $p$  of an unfractionated serum may be written

$$p = p_a + p_g + p_i \quad (1)$$

where  $p_a$  and  $p_g$  are the partial pressures due to albumin and globulins respectively, and  $p_i$  the ion pressure difference due to the excess of ions within the membrane (cf. Adair and Robinson<sup>11</sup>). In order to eliminate the effects of  $p_i$ , the comparison of the osmotic effects of different sera has to be made at infinite dilution of protein. This can be effected by determination of  $\Pi_0$  by extrapolation;  $\Pi_0$  being the limiting value of the ratio osmotic pressure/concentration ( $p/c = \Pi$ ) in an infinitely dilute solution. Fig. 2 shows the determination of  $1/\Pi_0$  for the above

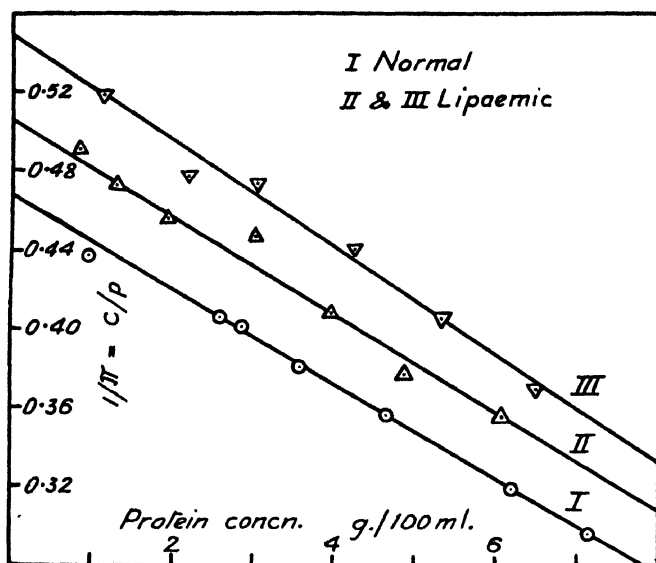


FIG. 2.—Relationship between protein concentration and  $1/\Pi (= C/p)$ .  
○ Observed values for normal; Δ and ▽ for lipaemic rabbit sera No. 1 and 2 respectively. The straight lines represent the equations:

- (I)  $1/\Pi = 0.467 - 0.0238C$   
(II)  $1/\Pi = 0.507 - 0.0250C$   
(III)  $1/\Pi = 0.550 - 0.0275C$ .

and

3 sera. The value of  $\Pi_0$  for the normal serum (curve I) was found to be 2.14, and for lipaemic sera (1) and (2), 1.97 and 1.82 respectively. The lipaemic sera, therefore, exerted a lower osmotic pressure than the normal serum.

Evidence on the question whether the observed differences in the osmotic pressures of the sera were due to their different lipid contents or to their different albumin-globulin ratios, may be obtained from the evaluation of the theoretical value of  $\Pi_0$ .

At infinite dilution the osmotic pressure of the serum may be expressed by

$$p = \frac{RT}{M_a} 10 C_a + \frac{RT}{M_g} 10 C_g, \quad (2)$$

<sup>11</sup> Adair and Robinson, *Biochem. J.*, 1930, **24**, 1864.

where  $C_a$  and  $C_g$  are the concentrations of albumin and globulins of the serum in g. per 100 ml. of solution;  $M_a$  and  $M_g$  the molecular weights of albumin and globulins respectively. If it be assumed that  $M_a = 69,000$  and  $M_g = 150,000$ , the value of theoretical  $\Pi_0$  at  $0^\circ$  is obtained from eqn. (3):

$$\Pi_0 = 2.464 X_a + 1.133 (1 - X_a), \quad (3)$$

where  $X_a = \frac{\text{g. albumin}}{\text{g. total protein}}$ , and hence  $(1 - X_a) = \frac{\text{g. globulins}}{\text{g. total protein}}$ .

For the normal serum  $X_a$  was found to be 0.675, and for lipaemic sera (1) and (2) 0.611 and 0.520 respectively. The theoretical values of  $\Pi_0$  for the 3 sera are therefore 2.031 (normal), 1.945 (lipaemic No. 1), and 1.824 (lipaemic No. 2), showing very good agreement with the observed values. It may be concluded, therefore, that the lower osmotic effects of the lipaemic sera were due to their higher globulin content as compared with the normal serum. It was found<sup>12</sup> that prolonged cholesterol feeding to rabbits produced regularly a decrease in serum albumin and an increase in globulins.

**Osmotic Pressures of Lipaemic Rabbit Serum before and after Extraction of Lipids.**—In order to obtain a definite evidence whether or not the lipids in serum exert an osmotic pressure, we investigated the colloid osmotic properties of a lipaemic rabbit serum before and after extraction of the lipids by the method of McFarlane.<sup>13</sup>

Table II records the lipid content of the lipaemic serum used for this experiment before and after extraction of the lipids. This shows that

TABLE II.—LIPID CONTENT OF POOLED LIPAEMIC RABBIT SERUM No. 3 BEFORE AND AFTER "DEFATTING"

	Before 'Defatting'	After 'Defatting'
	(Mg./g. Total Serum Protein)	
Neutral fat . . . . .	28	0.65
Phospholipids . . . . .	112	59.00
Cholesterol { free . . . . .	85	None
{ ester . . . . .	253	1.50
Fatty acids of cholesteryl esters . . . . .	182	0.98
Total lipid . . . . .	660	62.00

about 9/10ths of the lipid were extracted; most of the remaining 1/10th is accounted for by phospholipids of which only one-half was removed. This serum had the highest lipid content among the sera investigated (4.17 g. per 100 ml. of the unextracted serum).

Fig. 3 shows the osmotic pressure-concentration curves and Fig. 4 the determination of  $\Pi_0$  for this serum before and after "defatting" (continuous lines). It is evident that the serum in its lipaemic state exerted a higher osmotic pressure at finite protein concentrations than after extraction. On the other hand, the extrapolated value of  $1/\Pi_0$ , and hence the mean molecular weight of the osmotically active substances in the serum was the same before and after extraction. The observed value of  $\Pi_0$  was 1.923 (mean mol. wt. 88,500) and its theoretical value 1.873 ( $X_a = 0.556$  before and after extraction).

From the inspection of the continuous curves of Fig. 3 and 4 it is apparent, however, that from the point of view of osmotic effect at high protein concentrations there is a difference between a lipaemic and relatively

<sup>12</sup> Popják, (unpublished observations).

<sup>13</sup> McFarlane, *Nature*, Lond., 1942, 149, 439.

"fat-free" serum. We were able to show that this difference is due to the fact that in lipaemic sera the lipids occupy a considerable volume and thus reduce the solvent available to the proteins, with the result that the effective concentration of proteins is higher in the lipaemic than in the "fat-free" serum. When the corrections for volume occupied by proteins and lipids in the solution are made and protein concentrations are expressed as g. per 100 ml. of solvent, instead of g. per 100 ml. of solution, the difference between the osmotic pressure-concentration curves of lipaemic serum No. 3 before and after extraction is completely eliminated.

The partial specific volumes of unfractionated serum proteins and lipids were determined on highly concentrated sera. We found the specific volume of rabbit and human serum proteins (without water of hydration)

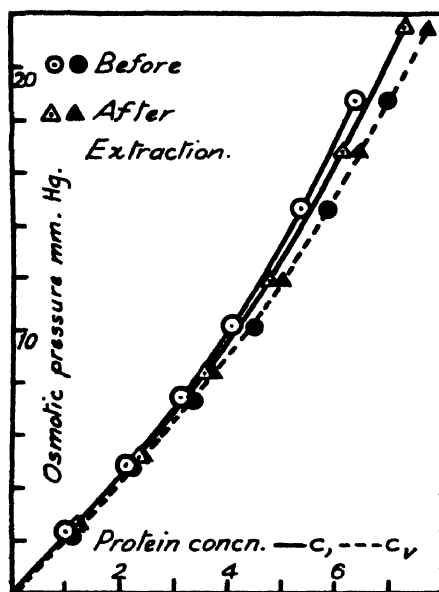


FIG. 3.—Relationship between protein concentration and osmotic pressure of lipaemic rabbit serum No. 3 before ( $\circ$  and  $\bullet$ ) and after ( $\Delta$  and  $\blacktriangle$ ) "defatting". Protein concentration is expressed as g. dry protein/100 ml. of solution ( $C$ ) for  $\circ$  and  $\Delta$ , and as g. dry protein/100 ml. of solvent ( $C_v$ ) for  $\bullet$  and  $\blacktriangle$ . The data for the continuous curves have been calculated by the equation  $p = \Pi_0 C / (1 - a \Pi_0 C)$ , and for the interrupted curve by

$$p' = \Pi_0 C_v / (1 - a' \Pi_0 C_v),$$

where  $a$  and  $a'$  are the slopes of the corresponding straight lines in Fig. 4.

to be 0.729 and 0.731 respectively, and that of serum lipids 0.996 cm.<sup>3</sup> g.<sup>-1</sup>. Thus the volume occupied by 1 g. of dry protein +  $x$  g. lipids per g. protein ( $v_{91}$ ) in the normal rabbit serum was 0.767, in lipaemic sera No. 1 and 2 1.074 and 1.206, and in lipaemic serum No. 3 before and after "defatting", 1.386 and 0.791 ml. respectively. The corrected concentration of the proteins,  $C_v$  (g. protein per 100 ml. of solvent) may be calculated by

$$C_v = C \times \frac{100}{100 + v_{91}C} \quad (4)$$

It can be seen from Fig. 3 (interrupted curve) that when the observed osmotic pressures of lipaemic serum No. 3, before and after "defatting", are plotted against  $C_v$ , the points fall within experimental error on a single curve. Similarly the values of  $1/\Pi_0 = C_v/p$  plotted against  $C_v$  are on

the same straight line (see Fig. 4, interrupted line), which can be expressed by the equation

$$1/\Pi_0 = 0.520 - 0.0213 C_0.$$

It follows that  $\Pi'_0$  (the limiting value of  $\Pi_0$  when  $C_0 \rightarrow 0$ ) is equal to  $1.923 = \Pi_0$ .

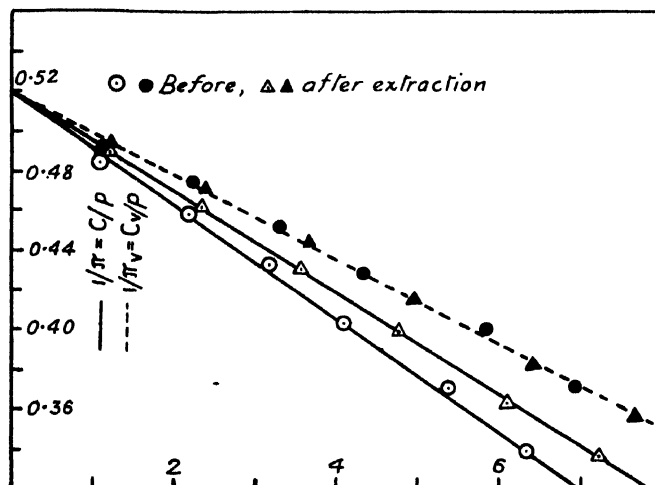


FIG. 4.—Relationship between protein concentration,  $C$  and  $C_0$ , and  $1/\Pi (= C/p)$  and  $1/\Pi_0 (= C_0/p)$  for lipaemic rabbit serum No. 3 before ( $\circ$  and  $\bullet$ ) and after ( $\Delta$  and  $\blacktriangle$ ) "defatting".  $\circ$  and  $\Delta$ ,  $1/\Pi$  plotted against  $C$ ;  $\bullet$  and  $\blacktriangle$ ,  $1/\Pi_0$  plotted against  $C_0$ . The straight lines represent the equations:

$$(I) \quad 1/\Pi = 0.520 - 0.0284C$$

$$(II) \quad 1/\Pi = 0.520 - 0.0255C$$

and

$$(III) \quad 1/\Pi_0 = 0.520 - 0.0212C_0.$$

**Osmotic Pressure Measurements on Normal and Nephrotic Human Serum.**—We have investigated a pooled sample of normal human serum and 3 sera obtained from patients suffering from subacute nephritis of the nephrotic type. The results obtained with one of the pathological

TABLE III.—LIPID CONTENT OF HUMAN SERA USED FOR OSMOTIC PRESSURE MEASUREMENTS

	Normal Serum	Nephrotic Serum
	(Mg./g. Total Serum Protein)	
Neutral fat . . . . .	19.5	77.0
Phospholipids . . . . .	33.0	70.0
Cholesterol { free . . . . .	5.3	23.5
ester . . . . .	20.0	48.5
Fatty acids of cholesteryl esters . . . . .	14.4	34.9
Total lipid . . . . .	92.0	244.0

sera only are recorded here, these being typical of the others. The lipid contents of the normal and nephrotic serum are shown in Table III.

The essential data are presented in Fig. 5 and 6 from which it can be seen that the nephrotic serum exerted a much lower osmotic pressure than the normal serum; the extrapolated value of  $\Pi_0$  for the former

being 1.515 and for the latter 2.020. The albumin content of the nephrotic serum was 32.3 % of the total proteins ( $X_a = 0.323$ ), and that of the normal serum 68.50 % ( $X_a = 0.685$ ). The theoretical value of  $\Pi_0$ , calculated by formula (3) is 1.567 for the nephrotic serum and 2.05 for the normal serum.

We could not find, therefore, that nephrotic sera exert a higher osmotic pressure than do normal sera. In fact the observed osmotic pressures were what one would anticipate from the low albumin content of nephrotic sera.

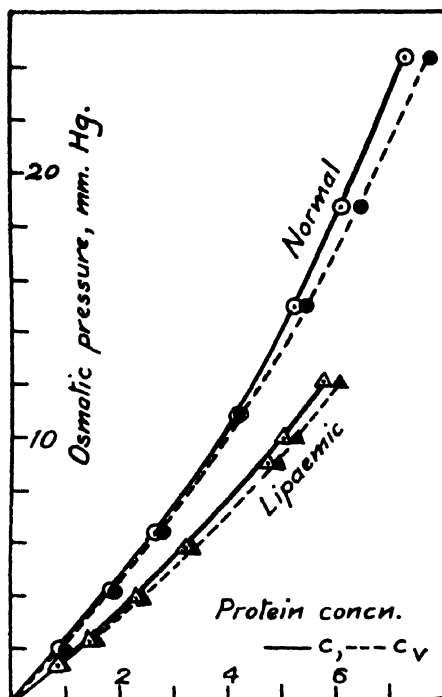


FIG. 5.—Relationship between protein concentration and osmotic pressure for normal ( $\circ$  and  $\bullet$ ) and nephrotic ( $\triangle$  and  $\blacktriangle$ ) human sera.  $\circ$  and  $\triangle$ , observed osmotic pressures plotted against  $C$ ;  $\bullet$  and  $\blacktriangle$ , observed osmotic pressures plotted against  $C_v$ . The data for the continuous curves have been calculated by the equation  $p = \Pi_0 C / (1 - a \Pi_0 C)$  and for the interrupted ones by  $p' = \Pi_0 C_v / (1 - a' \Pi_0 C_v)$ .

### Discussion

We believe that our results prove conclusively that the lipids do not exert an osmotic pressure. Our experimental sera contained, moreover, quantities of lipids that are rarely met with in man under pathological conditions.

It is difficult to reconcile our results with those of previous investigators. The discrepancies might be due to technical differences of osmotic pressure measurement. For example, Macheboeuf, Macheboeuf and Sandor did not really measure osmotic pressure, but only the degree of dilution of sera during dialysis. Obviously such a dilution would be influenced by a number of factors other than osmotic effects due to proteins. The comparison of the osmotic pressures between normal and lipaemic sera were made by Fishberg<sup>3</sup> and Rabinowitch<sup>4</sup>

at high protein concentrations only. Such a comparison might be misleading owing to the ion pressure difference (cf. Adair and Robinson<sup>11</sup>). It seems likely, however, that the higher osmotic effects of lipaemic sera reported by Fishberg and by Rabinowitch were due to the "volume effect" of the lipids which we observed. In a letter written to us Dr. Rabinowitch accepted this explanation and therefore we infer that he has withdrawn the claim that cholesterol exerts an osmotic pressure.

In a recent publication Oncley, Scatchard and Brown,<sup>14</sup> in order to explain certain anomalous results with an  $\alpha_1$ - and  $\beta_1$ -lipo-protein,

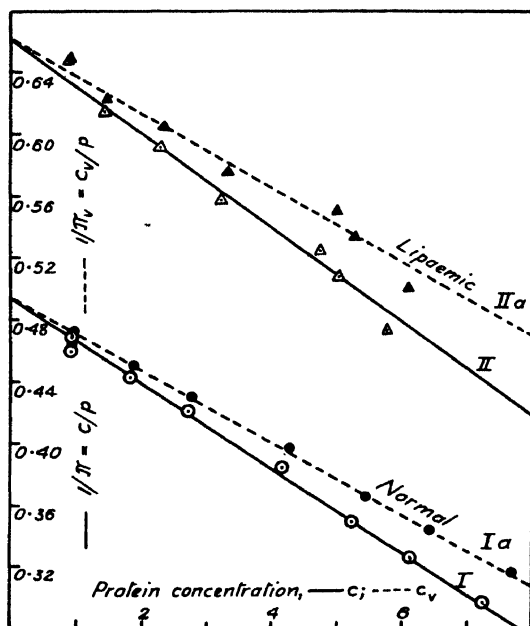


FIG. 6.—Relationship between protein concentration,  $C$  and  $C_0$ , and  $1/\Pi (= C/p)$  and  $1/\Pi_0 (= C_0/p)$  for normal ( $\odot$  and  $\bullet$ ) and nephrotic ( $\triangle$  and  $\blacktriangle$ ) human sera.  $\odot$  and  $\triangle$ :  $1/\Pi$  plotted against  $C$ ;  $\bullet$  and  $\blacktriangle$ :  $1/\Pi_0$  plotted against  $C_0$ . The straight lines represent the equations:

$$(I) \quad 1/\Pi = 0.495 - 0.0280C$$

$$(Ia) \quad 1/\Pi_0 = 0.495 - 0.0236C_0$$

$$(II) \quad 1/\Pi = 0.660 - 0.0304C$$

$$(IIa) \quad 1/\Pi_0 = 0.660 - 0.0238C_0.$$

and

assumed that lipids exert an osmotic pressure. They obtained a lower molecular weight for these two lipo-proteins by osmotic pressure measurements than by the ultracentrifuge, and have made the assumption that the higher osmotic pressures were probably due to lipids in equilibrium with the lipo-protein. We have examined the osmotic pressure data of Oncley *et al.* and conclude that the anomalous results are not due to osmotic pressure exerted by the lipids, but to a marked dissociation of the protein into smaller units.

We think it justifiable to conclude further that the lipids in serum are only in a loose combination (physical adsorption?) with the

<sup>14</sup> Oncley, Scatchard and Brown, *J. Physic. Chem.*, 1947, **51**, 184.

proteins. McFarlane's method of shaking the protein solution with ether and then freezing to below  $-25^{\circ}$  effects an almost complete extraction of lipids without denaturing the proteins and it does not appear likely that such a method could break a firmer, chemical bond. We would like to suggest that before a claim is made that lipids associated with proteins exert an osmotic pressure, a comparison should be made between the osmotic pressure of the "lipo-protein" before and after extraction of lipids and also the "volume effect" of lipids that we have described should be considered. These precautions would avoid any ambiguity in interpretation of results and may serve to clarify the definition of "lipo-proteins".

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#### GENERAL DISCUSSION\*

**Prof. M. Macheboeuf** (*Paris*) said: I have never spoken of the osmotic pressure of whole serum, but only of the osmotic pressure of serum albumins. The experimental results of Dr. Popjak and Dr. McCarthy are in complete agreement with ours. We have not spoken of  $\Pi_0$ , which is a property of extremely dilute solutions, but of pressures measured at concentrations close to those encountered under physiological conditions. Under these conditions lipids have a marked effect upon the osmotic pressure of albumins, as we have shown. Dr. Popjak and Dr. McCarthy have confirmed our measurements, and have put forward an explanation for the observation we made. Their work indicates that the lipids may act indirectly due to their volume. This interpretation is very interesting.

**Dr. A. S. McFarlane** (*London*) said: One implication of the osmotic inactivity of the plasma lipids must surely be that the molecular or micellar size of the lipo-proteins must be very small, incorporating not more than a few protein molecules in each particle. If larger numbers are involved, a reduction in the number of osmotically effective protein molecules in the plasma should become apparent.

\* On preceding paper.

## LIPO-PROTEINS IN THE PRECIPITIN REACTION

### THE EFFECT OF NINHYDRIN

BY F. TAYEAU, F. FAURE,\* E. NEUZIL AND R. PAUTRIZEL

*Received 2nd June, 1949*

Precipitating immune sera lose their flocculating properties when deprived of their lipids. Cholesterol does not play any part in the flocculating properties. The precipitating ability of fat-free immune rabbit serum is restored by treatment with ninhydrin at low concentration. This does not occur with horse immune serum. A physico-chemical interpretation of this phenomenon is presented.

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The lipids of lipo-proteins of immune sera play an important part in the specific precipitin reaction. Most immune sera, treated by a technique which removes the lipids but does not denature proteins,<sup>1</sup> retain their ability to combine with the corresponding antigens (first stage of antigen-antibody reaction), but lose their flocculating properties (second stage).<sup>2, 3</sup>

**The Different Lipid Fractions in the Specific Precipitin Reaction.**—Do the various lipids associated with the antibody play the same part in the flocculating property? By means of a technique described by one of us,<sup>4</sup> it is possible to remove cholesterol from serum quantitatively and specifically without removing the other lipids from proteins. Using this technique, we have been able to show that cholesterol does not interfere with the precipitating properties of the immune sera.<sup>5, 6</sup> Among blood lipids, phospholipids are probably the lipid fraction necessary for the formation of precipitates. It is interesting to compare this conclusion with those of Horsfall and Goodner.<sup>7</sup> These authors have shown that antipneumococcus horse or rabbit sera lose their precipitating properties when fat-free, and recover them when phospholipids are added.

This last observation should not be extended to all precipitating systems; we have shown that a fat-free diphtheria antitoxin does not recover its flocculating properties even if we fix lecithin on to the antitoxin again by the technique of Hofer.<sup>8</sup> The nature of the lipid-protein binding is therefore important.

What is the mechanism by which phospholipids facilitate precipitation? Phospholipids decrease the solubility of antibody proteins and therefore increase their precipitation probably owing to their hydrophobic groupings brought about by polymethylenic non-polar chains of their fatty acids. In the same way, the water-insoluble serum euglobulins become soluble when fat-free<sup>9</sup> or simply when phosphatide-free.<sup>10</sup> The insolubility of the specific precipitate therefore would be a physico-chemical phenomenon.

**The Effect of Ninhydrin on Precipitating Rabbit Sera.**—If this hypothesis is right, it should be possible to restore the flocculating properties of a fat-free precipitating serum by a modification of the solubility of its antibodies. For this purpose, we require a reagent which does not denature the antibody-protein too much, and which does not alter its specific determinants.

Different reagents, such as ketene<sup>11</sup> and formol,<sup>12, 13</sup> have been investigated. These reagents block up the  $\text{NH}_2$  groups of proteins, but they considerably delay the specific precipitation, as they injure

<sup>1</sup> Hardy and Gardiner, *J. Physiol.*, 1910, **40**, 58.

<sup>2</sup> Hartley, *Brit. J. Expt. Pathol.*, 1925, **6**, 180.

<sup>3</sup> Linderström-Lang and Schmidt, *Compt. rend. Carlsberg*, 1930, **18**, 1.

<sup>4</sup> Tayeau, *Compt. rend.*, 1941, **212**, 575.

<sup>5</sup> Tayeau and Neuzil, *Compt. rend. Soc. Biol.*, 1946, **140**, 509.

<sup>6</sup> Tayeau, Neuzil and Pautrizel, *ibid.*, 1947, **141**, 191.

<sup>7</sup> Horsfall and Goodner, *J. Expt. Med.*, 1935, **62**, 485, and *J. Immunol.*, 1936, **31**, 135.

<sup>8</sup> Pautrizel, *Recherches sur la genèse et la réactivité des anticorps* (Drouillard, Bordeaux, 1948), p. 99.

<sup>9</sup> Macheboeuf and Tayeau, *Compt. rend. Soc. Biol.*, 1938, **139**, 1181.

<sup>10</sup> Tayeau, *Bull. Soc. Chim. biol.*, 1944, **26**, 293.

<sup>11</sup> Sander and Goldie, *Compt. rend. Soc. Biol.*, 1937, **126**, 295.

<sup>12</sup> Loiseleur, *ibid.*, 1942, **136**, 435.

<sup>13</sup> Neuzil and Pautrizel, *ibid.*, 1947, **141**, 186 and 188.



the immunological determinants. Ninhydrin does not show the same disadvantage.

Immunological research on this reagent has already been carried out. Dulière<sup>16</sup> studied the changes brought about by ninhydrin on antigenic proteins. Recently, Eggerth<sup>17</sup> has not only pointed out the chemical modifications, but also the physical modifications of ninhydrinized proteins. Furthermore, he showed that the titre of certain agglutinins was increased when they were treated with small quantities of this product.

In former researches,<sup>18</sup> we proved that if we carry out the ninhydrization of a precipitating immune rabbit serum in such a way that no precipitation of any protein fraction occurs (0.1 % at 37° during half an hour), its rate of flocculation is considerably increased.

Besides, ninhydrin at low concentration decreases the solubility of rabbit antibody protein. As the titre of the antibody remains the same though the rate of flocculation is increased, we assume that ninhydrin at the previous concentration does not injure sensibly the "determinant groupings". As we noticed that ninhydrin is able to change the solubility of a fat-free antibody protein, we then wondered whether a ninhydrinized fat-free antibody protein recovers its lost flocculating ability.

The flocculating experiments were carried out by means of the  $\alpha$ -titration<sup>19</sup> with the following mixtures :

- (a) Rabbit anti-horse serum, horse serum (standard).
- (b) Fat-free rabbit anti-horse serum, fat-free horse serum.
- (c) Fat-free rabbit anti-horse serum treated with ninhydrin, fat-free horse serum.

The following results were observed :

- (a) Flocculation at the optimal proportion point in 48 min.
- (b) No flocculation.
- (c) Flocculation at the same optimal proportion point in 15 min.

Many identical experiments performed with sera from different rabbits gave the same results.

This phenomenon is really a specific precipitation, for if we use a ninhydrinized non-immune fat-free serum instead of a ninhydrinized immune fat-free serum, no flocculation occurs. These experiments therefore show that ninhydrin at a concentration of 0.1 % is able to restore the flocculating property to a fat-free immune rabbit serum without shifting the antibody titre.

In the former experiments, the antigen (horse serum) was, in fact, a complex mixture. A new series of experiments have been performed with different purified antigens, still using rabbit serum as the antibody. The results given by this new series of experiments (Table I) agree with our previous conclusion.

All these experiments agree with our first results and show that ninhydrin is able to restore the flocculating property to a fat-free rabbit immune serum.

<sup>16</sup> Schwartz, *Z. physiol. Chem.*, 1900, **31**, 460.

<sup>18</sup> Mascré and Herbain, *Bull. Soc. Chim. biol.*, 1930, **12**, 978.

<sup>16</sup> Dulière, *Compt. rend. Soc. Biol.*, 1938, **127**, 1122.

<sup>17</sup> Eggerth, *J. Immunol.*, 1941, **42**, 199 ; 1942, **45**, 303.

<sup>18</sup> Tayeau, Faure, Neuzil and Pautrizel, *Compt. rend.* (in press).

<sup>19</sup> Dean and Weeb, *J. Path. Bact.*, 1926, **29**, 473.

Rabbit anti-ovalbumin and rabbit anti-horse serum-albumin sera behave in a somewhat different way, for when fat-free, they do not lose completely their flocculating ability; however, the velocity of precipitate formation is considerably delayed. Ninhydrin, reacting with those two fat-free sera, considerably hastens the rate of flocculation.

Due to the fact that ninhydrinized immune proteins recovered their specificity and their precipitating titre, we furthermore show that the technique of Hardy and Gardiner<sup>1</sup> (used in these experiments for taking away lipids from the sera) does not injure the determinant groupings of the precipitins.

TABLE I

	Flocculation Time at the Optimal Proportion Point			
	Diphtheria Toxin ( $\beta$ -titration)	Sheep Pseudo-globulin ( $\alpha$ -titration)	Ovalbumin ( $\alpha$ -titration)	Horse Serum Albumin ( $\alpha$ -titration)
Antigen, antibody (standard)	2 hr.	10 min.	1 hr.	8 min.
Fat-free antigen, fat-free antibody	No flocculation	No flocculation	5 hr.	50 min.
Fat-free antigen, fat-free ninhydrinized antibody	10 hr.	14 min.	2 hr.	5 min.

**The Effect of Ninhydrin on Horse Immune Serum.**—Is it possible to extend this property to all the immune sera, regardless of their origin? We carried out experiments on horse immune serum by means of  $\beta$ -titration,<sup>20</sup> with the following mixtures:

- Horse antidiphtheria serum, diphtheria toxin.
- Fat-free horse antidiphtheria serum, diphtheria toxin.
- Fat-free horse antidiphtheria serum treated by ninhydrin, diphtheria toxin.

The following results have been observed:

- Flocculation in the optimal proportion point in 37 min.
- No flocculation; cloudiness after 24 hr.
- No flocculation; no cloudiness.

A new series of experiments performed with antidiphtheria and anti-tetanus sera from different horses gave similar results.

This result is not surprising, as our previous work<sup>18</sup> showed that ninhydrin delayed the velocity of flocculation of horse antidiphtheria serum—diphtheria toxin system. Let us recall here that ninhydrin, on the contrary, accelerates the rate of flocculation of the rabbit antidiphtheria system.

As we noticed that the concentration of ninhydrin played an essential part on the flocculating properties of the rabbit immune serum, we tried a new series of experiments in horse immune serum with varying amounts of ninhydrin. In no case were we able to give back the flocculating property to the fat-free serum.

<sup>20</sup> Ramon, *Compt. rend. Soc. Biol.*, 1922, 86, 711.

### Discussion

How can we explain the differences of action of ninhydrin upon rabbit and horse immune sera? It may be assumed that antibodies have different solubilities when they are elaborated by different zoological species. It could be due to the fact that the antitoxic fraction of horse serum is located in the pseudoglobulin fraction,<sup>21</sup> while the rabbit corresponding fraction is mostly located in the euglobulin fraction.

We have verified these data on the sera of the animals used in our own experiments; we have observed that horse antidiphtheria serum holds its precipitins in the pseudoglobulin fraction, while rabbit antidiphtheria precipitins are equally located in the pseudoglobulin and euglobulin fractions. Thus, for the same antigen, horse precipitins are more water-soluble than rabbit precipitins. Furthermore, this difference is much increased by the action of ninhydrin: under our experimental conditions, the solubility of ninhydrin-treated euglobulins is sensibly more decreased than the solubility of ninhydrin-treated pseudoglobulins.<sup>22</sup>

Our experiments show that under the influence of a chemical substance a fat-free immune serum is able to recover its lost flocculating properties. This conclusion proves that non-specific factors play the essential part in the second stage in the antigen-antibody reaction.

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<sup>21</sup> Seng, *Z. Hyg. Infekt.*, 1899, 31, 513.

<sup>22</sup> Unpublished data.

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## LIPO-PROTEINS IN SEROLOGY AND THE ROLE OF LIPID HAPTENES

BY MARY C. PANGBORN

*Received 20th May, 1949*

Lipids may function specifically as haptens in serologic reactions but they may also give rise to non-specific effects owing to their capacity of forming various types of complexes with proteins. Some of these non-specific effects are important only as sources of error but others play an essential if secondary role in the demonstration of immunologically specific reactions. The field is reviewed briefly and some applications of these principles in the serology of syphilis and tuberculosis are discussed.

The ability of lipids to form a variety of complexes with proteins introduces a number of special problems in serology. I hope it may be useful to gather together certain aspects of this general subject for critical discussion. In most cases the actual lipo-proteins involved have not been separated for study and the discussion must therefore centre on the behaviour of isolated lipids.

In certain instances it has been shown that blood lipo-proteins

play a definite part in serologic reactions. Hartley<sup>1</sup> found that removal of lipids by treatment with cold alcohol and ether, under such conditions that the proteins were not denatured, prevented flocculation of diphtheria toxin-antitoxin mixtures and of horse serum with anti-horse rabbit serum. Syphilitic sera freed from lipids in this way no longer fixed complement with Wassermann antigen. Horsfall and Goodner<sup>2</sup> extracted Type I anti-pneumococcus horse and rabbit sera by a similar technique and found that the property of precipitating homologous polysaccharides and agglutinating pneumococci was lost (horse) or greatly diminished (rabbit). The extracted serum still protected mice against infection and its *in vitro* combination with antigen was demonstrated by an inhibition method.<sup>3</sup> The precipitating and agglutinating properties were restored by admixture of traces of lipids: in horse serum the essential added lipid was lecithin while in rabbit serum it was cephalin. Thus a labile and easily reconstituted lipo-protein bond may be essential for the secondary or aggregation stage of the immune reaction, even though not needed for the combination of antigen with antibody.

A special case of the effect of a serum lipo-protein is described in the reports of Neurath and his collaborators.<sup>4-7</sup> These authors showed that globulin fractions from syphilitic and biologic false positive sera differed in that a serum fraction remaining in solution when the antibody globulins were precipitated inhibited the reaction between antigen and "false positive" globulin while it had little or no effect on the reaction with syphilitic antibody. The action of this inhibitor was due to combination with the antigen rather than with the antibody.<sup>5</sup> The inhibitor was found in the lipo-protein fractions of serum<sup>6</sup> and recently Volkin<sup>7</sup> has traced the inhibitor property to a lipid, apparently lecithin or some closely associated impurity.

When antigens composed wholly or in part of lipids are employed in serologic tests, the ability of the lipids to combine with serum proteins may give rise to a variety of non-specific effects which greatly complicate the study of any true antigen-antibody reactions that may be occurring in the same system. This is true of both complement-fixation and flocculation reactions.

In view of the multiple nature of complement and the fact that inhibition of any one of the four components is sufficient to destroy complement function,<sup>8</sup> it is to be expected that anticomplementary properties of antigens or sera may be due to a variety of unrelated causes. One case of an anticomplementary lipid—cephalin—has received particular attention. Wadsworth, Maltaner and Maltaner<sup>9</sup> showed that the anticomplementary activity of cephalin might be either reversible or irreversible; under certain conditions, reactivation could be brought about by adding  $\text{CaCl}_2$  in amounts equivalent to the cephalin added. These authors also showed that there was a

<sup>1</sup> Hartley, *Brit. J. Expt. Path.*, 1925, **6**, 180.

<sup>2</sup> Horsfall, Jr., and Goodner, *J. Expt. Med.*, 1935, **62**, 485.

<sup>3</sup> Horsfall, Jr., and Goodner, *J. Immunol.*, 1936, **31**, 135.

<sup>4</sup> Neurath, Volkin, Erickson, Craig, Putnam and Cooper, *Amer. J. Syph.*, 1947, **31**, 347.

<sup>5</sup> Volkin, Neurath and Craig, *ibid.*, 1947, **31**, 413.

<sup>6</sup> Volkin, Neurath, Erickson and Craig, *ibid.*, 1947, **31**, 397.

<sup>7</sup> Volkin, *J. Immunol., Virus Res., Expt. Chemotherapy*, 1949, **61**, 143.

<sup>8</sup> Pillemer, *Chem. Rev.*, 1943, **33**, 1.

<sup>9</sup> Wadsworth, Maltaner and Maltaner. *J. Immunol.*, 1936, **30**, 417.

quantitative parallelism between the activities of cephalin in inhibiting complement and in accelerating blood clotting, and Maltaner<sup>10</sup> has pointed out that the apparent fixation of complement by certain tissue extracts with normal rabbit sera was suspiciously parallel to the enhancement of the thromboplastic activity of the extracts by the same sera. These relationships have usually not been adequately considered as possible sources of error in studies of the antigenicity of lipids.

Anticomplementary effects may also be observed with other fractions of tissue lipids. The mechanisms involved are unknown. In attempting to circumvent this difficulty one comes on another as yet unexplained phenomenon—what one might call the “counter-anti-complementary” effect of lecithin. Various observations have been reported on the intensification of specific reactions<sup>11, 12</sup> and the elimination of anticomplementary effects<sup>13, 14</sup> by added lecithin. Some special cases of this will be discussed presently. Here it may simply be pointed out that the effect of lecithin in preventing anticomplementary activity of other lipids seems to be rather general and it deserves study in any problem where difficulties due to anticomplementary properties of lipids are encountered.

Because of the widespread use of flocculation reactions for the sero-diagnosis of syphilis, the possibility of non-specific serum precipitation by lipids is of great practical importance. Mackie and Anderson<sup>15</sup> and Anderson<sup>16</sup> studied precipitation by a number of different lipid preparations with normal sera of various species, and found that by employing varying ranges of serum dilutions and different temperatures of heating the serum they could obtain instances of flocculation with a wide variety of lipids. They were able to differentiate several types of precipitation according to dilution range and thermostability of the serum factor. Kahn<sup>17</sup> was able regularly to produce flocculation with Kahn antigen in “Kahn-negative” sera by varying the electrolyte content and the temperature of reaction. Such flocculates differed from the specific Kahn precipitates in that they were readily dissolved by increasing the concentration of NaCl. Kahn describes this low-temperature low-electrolyte flocculation as a “universal serologic reaction.” Such a term seems rather confusing. Rather, the “universal reaction” appears to be one more instance of non-specific lipo-protein combination, and illustrates the difficulty of drawing a sharp distinction between such combinations and those related to true immune phenomena. That such a distinction can be successfully drawn in practice, when all the conditions of precipitation are narrowly defined and rigidly controlled, is shown by the satisfactory degree of specificity observed in various flocculation tests for syphilis. It needs to be emphasized, however, that the basis for defining the conditions of such tests is still strictly empirical.

As incitants of specific antibodies, the lipids are peculiar in that, as pointed out by Landsteiner,<sup>18</sup> the alcohol-soluble haptenes and

<sup>10</sup> Maltaner, *Proc. Soc. Expt. Biol. Med.*, 1946, **62**, 302.

<sup>11</sup> Dienes and Scheff, *J. Immunol.*, 1926, **12**, 123.

<sup>12</sup> Freund, *ibid.*, 1927, **13**, 161.

<sup>13</sup> Schwab, *Z. Immunitäts*, 1936, **87**, 426.

<sup>14</sup> D'Alessandro and Greco, *ibid.*, 1938, **94**, 147.

<sup>15</sup> Mackie and Anderson, *J. Path. Bact.*, 1937, **44**, 603.

<sup>16</sup> Anderson, *Biochem. J.*, 1938, **32**, 282.

<sup>17</sup> Kahn, *Amer. J. Pub. Health*, 1947, **37**, 283.

<sup>18</sup> Landsteiner, *The Specificity of Serological Reactions* (Harvard University Press, Cambridge, Massachusetts, 1945), revised ed., p. 113.

chemically-known lipids are the only group of substances known to acquire antigenicity by simple admixture with proteins. The classical example of the method was the work of Landsteiner and Simms<sup>19</sup> on the alcohol-soluble Forssman antigen. These authors showed that animals injected with a mixture of alcohol-soluble haptene and a foreign protein, such as the serum of another species, may develop antibodies to the haptene component as well as the expected antibodies to the foreign protein. Subsequently, the same method was applied by numerous workers using more or less crude lipid extracts of tissues and of micro-organisms. The subject has been reviewed by Weil.<sup>20</sup> Where crude extracts have been used, the relationship of the resulting antibody to a lipid is clearly not proved, since such extracts always contain non-lipid substances. There are also serious disagreements as to the interpretation of some of the findings, due to differences in serologic techniques. For example, repeated reports of the antigenicity of cholesterol appeared in the early literature.<sup>21, 22</sup> The question was critically studied by Wadsworth, Maltaner and Maltaner<sup>23</sup> employing a carefully standardized quantitative complement-fixation technique<sup>24</sup> by which it could be shown that there was no direct proportionality between the amount of complement fixed by the cholesterol "antisera" and the amount of serum used, and in this respect the reaction differed sharply from those of known serologic specificity.

With all these qualifications, however, the existence of genuine lipid haptenes is well established. Two special cases will be discussed in some detail. In both these cases the chemical nature of the active lipids is reasonably well known and their specific serologic reactivity is beyond dispute; the two have, moreover, certain interesting points in common.

The use of lipid "antigens" in the serodiagnosis of syphilis is certainly the most widely known application of lipid reagents in serology. In spite of the lack of any apparent connection between the test reagent and the disease, it seems to be generally agreed that the reacting substance in syphilitic sera behaves like a true antibody, whether it is produced by some sort of auto-immunization or whether the incitant is a spirochaetal antigen that just happens to cross-react with a component of normal tissue; this controversy has been recently reviewed by Davis.<sup>25</sup> The tissue component, the essential active component of the antigen, was isolated from beef heart and identified as a complex phosphatidic acid, cardiolipin.<sup>26, 27</sup> In the tissues it is presumably combined with protein. Furth and Kabat<sup>28</sup> found that the "Wassermann" activity of saline extracts of beef heart was associated with a heavy particle substance sedimentable in the ultracentrifuge. Demonstration of *in vivo* antigenicity by immunization of animals with crude "Wassermann substance" combined with protein was apparently

<sup>19</sup> Landsteiner and Simms, *J. Expt. Med.*, 1923, **38**, 127.

<sup>20</sup> Weil, *Bact. Rev.*, 1941, **5**, 293.

<sup>21</sup> Sachs and Klopstock, *Biochem. Z.*, 1925, **159**, 491.

<sup>22</sup> Weil and Besser, *Z. Immunitäts.*, 1932, **76**, 76.

<sup>23</sup> Wadsworth, Maltaner and Maltaner, *J. Immunol.*, 1935, **29**, 135.

<sup>24</sup> Wadsworth, Maltaner and Maltaner, *ibid.*, 1931, **21**, 313.

<sup>25</sup> Davis, *Medicine*, 1944, **23**, 359.

<sup>26</sup> Pangborn, *J. Biol. Chem.*, 1942, **143**, 247.

<sup>27</sup> Pangborn, *ibid.*, 1947, **168**, 351.

<sup>28</sup> Furth and Kabat, *Science*, 1941, **94**, 46.

successful.<sup>29, 30</sup> Such experiments have not yet been repeated with cardiolipin.

The use of cardiolipin in diagnostic tests for syphilis depends for success on a balance between specific reactivity and non-specific colloidal properties. It therefore illustrates a number of the general points already mentioned.

Thus it was soon found that cardiolipin alone could not be used as an antigen either in complement-fixation or flocculation tests. The possibilities of demonstrating its combination with antibody by inhibition or absorption methods have not yet been explored. The practical problem of preparing useful diagnostic antigens was solved by admixture of purified lecithin and cholesterol. The effects of various proportions of these substances were first carefully studied by Maltaner and Maltaner<sup>31</sup> and by Brown,<sup>32, 33</sup> and applications of the same principle in different serologic techniques were reported by others.<sup>34-36</sup> Cardiolipin alone was anticomplementary but this effect was completely inhibited by addition of five parts of lecithin.<sup>31</sup> Mixtures of cardiolipin and lecithin reacted in both complement-fixation and flocculation tests, while addition of cholesterol further increased the sensitivity of the reactions. Two interesting points about the properties of these mixtures may be noted: (i) the amount of cardiolipin in the optimally reacting mixture is small in proportion to the amounts of the presumably non-specific ingredients, and (ii) specificity as well as sensitivity depends on the proportions of the three components. Thus a complement-fixation antigen containing insufficient lecithin may tend to give non-specific reactions. Moreover, even with optimally adjusted mixtures of the three components, some false positive reactions are still obtained. The use of cardiolipin antigens makes it possible to differentiate "false positive" reactions that are related to antigen impurities from those that depend on quite different factors, such as the presence in certain sera of the reacting globulins studied by Neurath and his colleagues.<sup>4-7</sup> These authors found that syphilitic and false positive globulins could be more sharply differentiated by the reaction of the inhibitor with cardiolipin than when crude antigens were used.<sup>5</sup>

The best known instance of a lipid antigen from a bacterial source is the phosphatide of the tubercle bacillus. Since Anderson's first work on the separation of this substance,<sup>37</sup> there have been several studies of the serologic properties of more or less purified tubercle bacillus lipids.<sup>38-41</sup> In these reports there is complete agreement that

<sup>29</sup> Sachs, Klopstock and Weil, *Deut. Med. Wchnschr.*, 1925, **51**, 589.

<sup>30</sup> Eagle, *J. Expt. Med.*, 1932, **55**, 667.

<sup>31</sup> Maltaner and Maltaner, *J. Immunol., Virus Res., Expt. Chemotherapy*, 1945, **51**, 195.

<sup>32</sup> Brown, *ibid.*, 1946, **52**, 17.

<sup>33</sup> Brown, *ibid.*, 1946, **53**, 171.

<sup>34</sup> Kline, *Amer. J. Clin. Path.*, 1946, **16**, 68.

<sup>35</sup> Harris, Rosenberg and Riedel, *J. Ven. Dis. Inf.*, 1946, **27**, 169.

<sup>36</sup> McDermott and Kahn, *J. Lab. Clin. Med.*, 1948, **33**, 1224.

<sup>37</sup> Anderson, *J. Biol. Chem.*, 1927, **74**, 525, 537.

<sup>38</sup> Pinner, *Amer. Rev. Tuberc.*, 1928, **18**, 497.

<sup>39</sup> Doan, *Proc. Soc. Expt. Biol. Med.*, 1929, **26**, 672.

<sup>40</sup> Pedersen-Bjergaard, *Z. Immunitäts.*, 1934, **82**, 258.

<sup>41</sup> Macheboeuf, *et al.* (a) *Compt. rend.*, 1937, **204**, 1843; (b) *ibid.*, 1939, **209**, 700; (c) *Bull. Soc. Chim. biol.*, 1934, **16**, 355; (d) *ibid.*, 1935, **17**, 1194, 1201, 1210.

the tuberculophosphatide fixes complement with tuberculous sera and that it represents the chief active ingredient of antigenic extracts made with lipid solvents. Pinner<sup>38</sup> and Doan<sup>39</sup> reported that the Anderson phosphatide could be used for precipitation as well as complement-fixation tests. Macheboeuf<sup>41</sup> found no precipitating activity in his purified phosphatide and considered any such activity to be due to a polysaccharide contaminant. Pinner,<sup>38</sup> Doan,<sup>39</sup> Pedersen-Bjergaard,<sup>40</sup> and Chargaff and Schaefer<sup>42</sup> all reported that phosphatides prepared by Anderson's method stimulated antibody production in animals—differing therefore from other lipids in not requiring prior admixture with protein. Again, Macheboeuf's preparation differed; it was not antigenic but strictly a haptene. Some of these discrepancies might be due to varying serologic techniques as well as to differences in purity.

A renewed serologic study of this substance has recently been undertaken and while it is too soon to say much about it certain points of comparison with the better known cardiolipin system are suggestive. Cardiolipin and the tuberculophosphatide can be classified together chemically, since both are salts of complex phosphatidic acids. Both are sufficiently complex molecules to provide ample opportunity for uniqueness of chemical structure such as is associated in other cases with serologic specificity. Finally, the effect of lecithin on the reaction of these two substances with complement is similar. Preliminary study of a partly purified BCG phosphatide<sup>43</sup> showed that it was decidedly anticomplementary but addition of three parts of lecithin obscured the anticomplementary effect while not altering the reactivity with immune serum. Such results lead to the hope that some of the principles successfully applied in the study of the syphilis antigen-antibody system may prove useful in developing an improved antigen for tuberculosis.

To recapitulate, we have seen that lipids may take part in serologic phenomena by either specific or non-specific reactions. These non-specific reactions may be mere sources of error or they may actually be essential in providing the physico-chemical conditions under which a specific reaction is manifested. Study of cardiolipin and of the tuberculophosphatide indicates that non-specific activity (inhibition of complement) may even be a property of the same molecule that also has a truly specific reactivity. There seems to be no reason to doubt that serologic specificity of lipids, as of other antigens, is related to particular chemical configurations. It is evident, however, that such relationships are not so easy to bring out as in the case of polysaccharides and the various conjugated-proteins. Even with purified substances such as cardiolipin, demonstration of the specific reaction is narrowly conditioned by non-specific colloidal properties of the system.

ADDENDUM (14th November, 1949): After this paper was submitted, some additional observations on the phosphatide of the tubercle bacillus were made. The phosphatide was prepared from a recently isolated human strain of the bacillus and was purified in the form of the Ba salt until it contained 4.2 % phosphorus and no nitrogen. This method gives much better yields of a purified product than can be obtained by fractionation with solvents alone.

In studying the complement-fixing activity of the phosphatide, tuberculous horse serum was used, as has been commonly done by other

<sup>41</sup> Chargaff and Schaefer, *Ann. Inst. Past.*, 1935, 54, 708.

<sup>43</sup> Maltaner and Pangborn (unpublished).



investigators. It has not yet been determined whether the antigen is equally suitable for tests with human serum. The phosphatide was somewhat anticomplimentary alone but this property was eliminated by addition of 6 parts of lecithin, with some increase in the antigenic activity. The effect of added lecithin thus appeared qualitatively similar to that observed with cardiolipid although it was quantitatively much less.

It has been repeatedly observed that lipoidal extracts of the tubercle bacillus tend to react with syphilitic sera, and Debus<sup>44</sup> found this activity increased by addition of lecithin. A group of 100 syphilitic sera was tested with an antigen consisting of the purified tuberculophosphatide mixed with 6 parts of lecithin; in this preliminary series it appeared that there was no correlation between the titres of the sera with cardiolipid antigen and with tuberculophosphatide. Further study is necessary, however, before this point can be adequately clarified. The work is being continued.

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<sup>44</sup> Debus, *Z. Immunitäts*, 1931, **72**, 373.

#### GENERAL DISCUSSION\*

**Prof. F. Tayeau** (*Bordeaux*) said: I have already pointed out in my paper that the first stage of the precipitin reaction (binding of antigen and antibody) still exists when fat-free antibody are used (Hartley, Linderström-Lang and Schmidt.)

Two main theories are generally opposed with regard to the second stage:

(i) Some authors think that the binding of specific groups of antigen and antibody continues until the aggregates have a sufficient volume to sedimentate (Marrack, Heidelberger, Pauling.)

(ii) Others estimate that elementary antigen-antibody aggregates, small and water-soluble, clump together by the action of unspecific forces to form a precipitate (Haurowitz); the solubility of the large aggregates depends mostly on the hydrophilic and hydrophobic properties of the surface grouping. Our results seem to be an argument for this last theory.

There are sera which, when fat-free, do not completely lose their flocculating ability; for instance, some months ago Sandor in Paris isolated from anti-plague serum a euglobin fraction which is absolutely fat-free and which abundantly precipitates with his corresponding antigen. We are now carrying on systematic work on this question to get a better idea of the different behaviour of fat-free antibody when different antigens have been used for immunization.

**Prof. J. R. Marrack** (*London*) said: The experiments of Prof. Tayeau and his colleagues are strong evidence against the theory that the formation of precipitates by antigens and antibodies depends solely on specific forces. Experiments by Orlansky in my laboratory agree with those of Prof. Tayeau in showing the importance of the solubility of the antibody.

Neither  $\alpha$ - nor  $\beta$ -lipo-proteins are involved in these reactions, for precipitation occurs readily when antigen is added to  $\gamma$  globulin (free from faster components) isolated from antiserum. The question arises whether  $\gamma$  globulin contains enough lipid to affect its solubility appreciably.

In connection with Klotz's theory, mentioned by Prof. Luck, the forces by which antibodies (which are  $\gamma$  globulins) combine with antigens

\* On two preceding papers.

must be similar to those by which albumin combines with dyes. But antibodies do not differ from inert  $\gamma$  globulins in the number of  $-\text{COOH}$ ,  $-\text{NH}_2$ , and  $-\text{OH}$  groups that they contain.

**Prof. F. Haurowitz** (*Bloomington, Indiana, U.S.A.*) said: Prof. Tayeau's assumption that the second part of the precipitin reaction is non-specific is in agreement with our view (1938) based on the inhibition of the precipitation by neutral salts. Most probably the antigen-antibody complex consists of an antigen nucleus surrounded by many antibody molecules. The aggregation of such complexes is brought about by the same non-specific forces which are responsible for the insolubility of euglobulins at low ionic strength. Euglobulins, as well as antigen-antibody aggregates, are solubilized by neutral salts.

**Prof. E. Chargaff** (*New York*) said: At the concentration of ninhydrin used, are all the  $\text{NH}_2$  groups destroyed?

**Prof. F. Tayeau** (*Bordeaux*) said: At the concentration of 0.1 % (at 37° during half an hour) ninhydrin blocks 5 to 10 % of the free  $\text{NH}_2$  groups of proteins.

**Prof. M. Heidelberger** (*New York*) said: Our results are not in accord with the views expressed in Prof. Tayeau's paper, or in the Discussion. Removal of lipids from antisera certainly delays or inhibits gross precipitation, but if mixtures of antigen and antiserum are allowed to stand for adequate periods and are then centrifuged at 3,000 rev./min. more specific nitrogen is actually precipitated from delipidized sera than from the unextracted ones. The lipids therefore seem to facilitate gross particle formation, probably mechanically, from the small aggregates initially formed, as well as to prevent complete separation of antibodies.

**Prof. F. Tayeau** (*Bordeaux*) said: I am very interested by Prof. Heidelberger's experiments. The formation of gross insoluble particules is precisely what is generally called the second stage of precipitin reaction. I also agree with the fact that lipids play in this stage a non-specific action, probably mechanical. The centrifugation used by Prof. Heidelberger precipitates aggregates which, in generally used conditions, are dispersed in the aqueous medium.

**Prof. M. Heidelberger** (*New York*) asked: In view of the preceding can one say where the so-called first stage ends and the so-called second stage begins?

**Dr. A. Lasnitzki** (*Birmingham*) said: I should like to draw the attention of Prof. Tayeau to investigations I carried out many years ago in collaboration with Prof. Friedberger, the German immunologist.<sup>1</sup> Using rabbit antisera throughout, it had been shown in previous work that a marked precipitin reaction could frequently be obtained, not only with the homologous serum, but also with one or more sera from different species. For instance, an anti-bovine serum reacted with bovine as well as horse serum, an anti-cat serum with cat as well as dog serum. Morphologically the specific precipitate consisted of coarse particles having a spongy structure, while the unspecific precipitate was composed of fine, compact granules. Assuming that the former was essentially due to a reaction between proteins and the latter mainly to a reaction between lipids, the effect of the removal of lipids from anti-serum and serum (both homologous and heterologous) had been studied. The extraction was made with ether at room temperature. It was found that the unspecific reaction could be abolished, or at least considerably reduced, whereas the specific reaction usually was reduced to a minor degree, or appeared to be unchanged. As a rule the effect was less if only one of the two components had been treated with ether. We concluded from these results that lipids might play a significant part in the formation of the unspecific precipitate, but might likewise be of some importance in the formation of the specific one.

<sup>1</sup> Friedberger and Lasnitzki, *Biochem. Z.*, 1923, 137, 312.

**Prof. F. Tayeau** (*Bordeaux*) said : The extraction of serum by ether alone at room temperature removes a minute amount of lipids (Hoppe-Seyler). Furthermore, the physical properties of ether treated proteins are different from native ones (Lecomte du Noüy).

**Prof. M. Macheboeuf** (*Paris*) said : Miss Pangborn in her paper writes : " Some of these discrepancies might be due to varying serologic techniques, as well as to differences in purity." But Chargaff and Schaefer worked with me in the same department of the Institut Pasteur with identical serological techniques. The divergences, therefore, are due solely to some differences in purity. Anderson's phosphatide was a mixture.

I might also add that it was not Anderson who proved that the B.K. lipidic haptene was a phosphatidic acid. He believed that it was a *phosphatide*. It is with Mlle. M. Faure and Mme. G. Levy that we have been able to prove that it evolves from a phosphatidic acid, which was also Block's theory.

**Miss M. Pangborn** (*New York*) said : The sentence quoted from my paper by Prof. Macheboeuf refers to an entire paragraph in which the work of several authors other than those in Prof. Macheboeuf's laboratory are cited. There are significant differences in the serologic techniques used by these various workers.

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## RECENT STUDIES ON CELLULAR LIPO-PROTEINS

BY ERWIN CHARGAFF

*Received 8th May, 1949*

Lipo-proteins are outstanding examples of macromolecular conjugated proteins isolated from the cell under mild conditions. An attempt is made to classify these substances according to the types of lipid or of protein that they contain and to the cellular components from which they are derived. Examples of synthetic lipo-proteins (of the salt type) and of more complex naturally occurring substances are discussed. Among the latter, detailed attention is paid to the thrombo-plastic protein, to the chromo-proteins of plant cells, and to the lipo-nucleo-protein complexes of yeast.

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The problem of conjugation, and particularly that of conjugated proteins, has in the past few years acquired new importance in biochemistry. In fact, the formulation of the problem itself is of relatively recent date, as could be shown by tracing the birthdays of such terms as " prosthetic group " or " apoenzyme. " The text-book distinction, however, between simple and conjugated proteins has probably outlived its usefulness. It is not unlikely that most cellular proteins, in contrast to some proteins in extra-cellular fluids, exist in conjugation with many substances of non-protein character. To decide, which is the rider and which the horse, sometimes is not easy : there are fractions, e.g. in aqueous brain extract that would deserve the designation of proteo-lipids rather than lipo-proteins.

The more we learn about the composition of cells and biochemical systems in general, the clearer it becomes that many substances that appear in the test-tube as discrete and unconnected, are in the living organism united to larger, more complex, and more specific structures. Thus we encounter combinations between proteins and lipids, proteins

and carbohydrates, lipids and carbohydrates, etc. We would probably be guilty of no more than a mild distortion, were we to state that within the living cell everything is high-molecular. The ability of the living cell to retain easily diffusible substances against a concentration gradient has been a much discussed and baffling subject. While the explanation usually proffered, that it is through its metabolic activities that the cell is able to bring this about, probably is correct, the part that the structure of the cell plays in its metabolism must not be forgotten. It is impossible to write the history of the cell without attention to its geography.

Lipid-protein complexes, like most other conjugated proteins, often are quite unstable; they are not easy to handle and have, therefore, probably often remained unrecognized. Even now, our knowledge of how to treat them still is in its infancy. These complexes are widely distributed in living matter. They are almost ubiquitous within the cell, occurring in cell nuclei, mitochondria and other cytoplasmic inclusions, cell membranes, etc. Egg yolk is very rich in a lipo-protein, lipo-vitellin, and appears to contain other lipid complexes in addition. Similar compounds occur in fish eggs, e.g. ichthulin; but very little work has been done on these substances. The very important lipo-proteins found in extracellular fluids are not being considered here.

The term lipo-protein is used for compounds between proteins and lipids, which latter are defined as a group of naturally occurring fatty acid derivatives, soluble in organic solvents. A classification by the type of lipids or of proteins that they contain would be desirable, but is mostly impossible. One grouping by classes of proteins could be attempted, since lipo-proteins appear to represent associations with one of three main protein groups:

- I. Nucleoproteins (mostly of the pentose type).
- II. Phosphoproteins.
- III. Phosphorus-free proteins.

Group I comprises the cytoplasmic lipo-proteins among which the thromboplastic protein has, perhaps, been the most thoroughly investigated. To this group likewise seem to belong a particulate lipo-protein from yeast which will be mentioned later, the pigmented carotinoid-protein complexes from carrots, and possibly also the lipo-proteins of the chloroplasts. Group II includes the lipo-proteins from egg yolk and probably also from milk. To Group III belong the lipo-proteins of blood plasma.

A survey of the types of primary valency bonds available for linkage of lipids to proteins may be of interest. This is taken from a review article.<sup>1</sup>

1. Fatty acids: Carboxyl group, electrostatic (salt); covalent (ester, amide, etc.).
2. Triglycerides: None. (Mono- and diglycerides could, of course, form covalent ester links through their free hydroxyl groups.)
3. Lecithin: Trimethyl ammonium group, electrostatic (salt). Phosphoric acid group, electrostatic (salt); covalent (ester, etc.).
4. Cephalin: Amino group, electrostatic (salt); covalent (amide). Phosphoric acid group, electrostatic (salt); covalent (ester, etc.).

<sup>1</sup> Chargaff, *Advances in Protein Chemistry* (Academic Press, New York, 1944), Vol. I, p. 1.

5. Phosphatidyl serine: Amino group, electrostatic (salt); covalent (amide). Phosphoric acid and carboxyl groups, electrostatic (salt); covalent (ester, amide, etc.).
6. Sphingomyelin: Trimethyl ammonium group, electrostatic (salt). Phosphoric acid group, electrostatic (salt); covalent (ester). Hydroxyl group, covalent (ester).
7. Phrenosin and kersin: Hydroxyl groups, covalent (ester).
8. Xanthophyll and sterol esters: None.

The phosphatidic acids, glycerol ethers, and acetal phosphatides probably behave similarly to Groups 1, 2 and 4 respectively.

It is, of course, necessary to distinguish between genuine lipid complexes and mixtures or loose adsorption systems. The term lipo-protein connotes a group of compounds with properties (e.g. biological reactivity, solubility, colour, optical and other physical constants) different from those of the sum of their components. The inspection of the polar characteristics of lipo-proteins could lead to the following provisional classification:

- I. Covalent compounds (groups 1 and 3 to 7).
- II. Electrostatic compounds (groups 1 and 3 to 6).
- III. Secondary valency compounds (groups 1 to 8).

There is little evidence of the occurrence of covalent lipo-proteins (e.g. esters, amides, etc.) in nature. Electrostatic lipo-proteins are represented by salts, where the attraction must be due to ionic forces between the lipid and the protein. Certain synthetic lipo-proteins and lipo-protamines belong to this type. Such substances are stable within a certain pH range only. Therefore, there will be a significant difference in the reactivities of lecithin and sphingomyelin on the one hand (these are practically neutral lipids) and the more acidic phosphatides on the other. At the physiological pH, lecithin can hardly be expected to form salts with tissue proteins, whereas cephalin and especially phosphatidyl serine will be able to combine with protamines and with thymus histone, but not with globin, by means of ionic bonds to form insoluble products.<sup>2, 3</sup>

Most lipo-proteins occurring in nature probably have to be classified as secondary valency compounds. These complexes are stable, and move intact in an electric field, at a pH well above the isoelectric points of their component parts. It is not improbable that some of the lipids that are unable to attach themselves by electrostatic or covalent bonds could exist as solid solutions in the prosthetic lipid portion of a lipo-protein. It is very remarkable that the treatment with alcohol appears to disrupt the protein-lipid linkage. It must be said that, as far as biochemistry is concerned, the nature of the secondary valency or van der Waals' forces is largely unknown. It is quite possible that a new direction will have to be introduced into our conceptions of compound formation within the group of macromolecules. In a protein the average electrical charge, reflected in its isoelectric point and electrophoretic mobility, perhaps is not an indication of the specific spacing and localization of ionizable groupings. One could, for instance, conceive that a number of positive charges (e.g.  $\text{NH}_2$  groups) or negative

<sup>2</sup> Chargaff, *J. Biol. Chem.*, 1938, **125**, 661.

<sup>3</sup> Chargaff and Ziff, *ibid.*, 1939, **131**, 25.

charges (e.g. COOH groups) could be concentrated at certain critical spots in the protein. Thus a specific field of forces would be created in which discrete electrical charges could be so compressed as to create the possibility of the formation of electrostatic bonds; but only if certain spatial requirements were fulfilled. It may generally be assumed that any action resulting in the displacement or the distortion of such centres of attachment in the protein will bring about the cleavage of the conjugated protein. This does not necessarily disprove the essentially electrostatic nature of such macromolecular van der Waals' compounds. It goes without saying that hydrogen bonding probably will also be of great importance in the formation of these complexes; but no strict evidence has been put forward so far. In whatever manner, however, the structure of these conjugated compounds may be explained, it will be necessary to allow for the continuous opening and closing of bonds in which the whole is altered without being destroyed; one will have to take into account that peculiarly trembling balance through which the living cell struggles against the establishment of a permanent equilibrium.

As an example of what may be considered as a precursor of a cellular lipo-protein, the lipo-vitellin from hen's egg yolk may be mentioned.<sup>4</sup> The lipid-vitellin complex present in yolk has for many years been regarded as the classical instance of a naturally occurring lipo-protein, although it is by no means typical of the complexes found in tissues and plasma. The purified lipo-protein complex contains N 13.0, P 1.5, total lipids 23, and phosphatides 18 %. Only one-fifth of the yolk lipids, however, is bound in the conjugated protein; the remainder is extractable with ether. There appears to be no essential difference in the composition of the free and the bound phosphatides, nor could any difference be shown in the rates of formation of these lipids, when radioactive phosphorus was used as a marker. The bound and the free lipids appeared to be in equilibrium.<sup>5</sup>

In discussing lipo-proteins as part of the cell structure only brief reference can be made to their occurrence in cell nuclei and in membranes, since very little chemical information on these structures has come to light so far. That mitochondria and other cytoplasmic structures contain lipo-proteins has been shown by the work of Bensley, Claude and others. It may be hoped that the recent application of paper chromatography to the separation of the nitrogenous constituents of phosphatides<sup>6</sup> will prove useful to studies of this kind, the more so since it has recently been possible to develop methods, on this basis, for the quantitative estimation of choline, ethanolamine, serine, etc., as they occur in lipid hydrolyzates. (Unpublished work with Miss Celia Levine.)

One outstanding, long-recognized, and easily demonstrable feature of tissue extracts is their strong blood-clotting activity. For this reason, we have studied in considerable detail the lipo-proteins of tissue cells that are responsible for this effect. One interesting feature in this work is the possibility of following the purification of a lipo-protein and the reactions to which it may be subjected by biological assay methods. The best studied example is the thromboplastic protein of beef lung. Similar fractions were isolated from human organs (lung

<sup>4</sup> Chargaff, *J. Biol. Chem.*, 1942, **142**, 491.

<sup>5</sup> Chargaff, *ibid.*, 1942, **142**, 505.

<sup>6</sup> Chargaff, Levine and Green, *ibid.*, 1948, **175**, 67.

and placenta).<sup>7</sup> Minute amounts of a fraction resembling the tissue material could be isolated from human plasma.<sup>8</sup>

The place of this fraction in our current conception of the mechanism of blood coagulation seems clear: it catalyzes the conversion of prothrombin to thrombin. Recent work in this laboratory has proved that this activating effect is of enzymatic nature, since it was possible to recover the thromboplastic protein with undiminished activity following its action on prothrombin.<sup>9</sup> The same series of studies also revealed the profoundly disrupting effect of sodium desoxycholate on the thromboplastic protein.<sup>9, 10</sup>

Crude preparations of the thromboplastic protein can be obtained by fractional salt precipitation of tissue extracts.<sup>11</sup> Much better fractions are obtained, however, when extracts are fractionated by centrifugation at different speeds.<sup>12</sup> It may generally be said that the fractional centrifugation at a high speed probably is the best method for the isolation of those cytoplasmic lipo-proteins that are nucleoprotein complexes (Group I, mentioned above). If sufficient care is taken, preparations may be obtained which are homogeneous with respect to particle size; all purified fractions were found homogeneous in the electrophoresis cell.

Electron micrographs of the preparation revealed the presence, together with some aggregated material, of a large percentage of almost perfect spheres with a diameter of 80 to 120 m $\mu$ . The particles probably are highly hydrated, as the calculated frictional ratio  $f/f_0 = 1.41$  corresponds to an axial ratio of 8 for a prolate ellipsoid. The partial specific volume of the protein was very high,  $V_{27} = 0.87$ , as would be expected of a lipid-protein complex. The sedimentation constant  $s_{20} = 330 S$  and the diffusion constant  $D_{20} = 0.38 \times 10^{-7}$  correspond to a very high particle weight from rate of sedimentation, viz. 167 million. The electrophoretic mobility at pH 8.6 was 8.4 cm.<sup>2</sup> volt<sup>-1</sup> sec.<sup>-1</sup>  $\times 10^{-5}$ .

The treatment with hot alcohol-ether resulted in the extraction of 40-45 % of lipids. Following the removal of the lipids, the protein residue contained N 13.4, P 0.4, amino sugar 1 %. The residual phosphorus seems mostly due to the presence of a pentose nucleic acid which can, at least in part, be split off by heating. It had an absorption maximum at 2610 Å.

The spectrum of the intact thromboplastic protein in borate buffer (pH 7.9) has been published.<sup>9</sup> It showed a plateau around 2600 Å, undoubtedly due to the presence of nucleic acid, and a maximum at 2250 Å, which latter disappeared on storage.

The disintegration of tissue lipo-proteins is not easy to achieve, and its mechanism little understood. Ether alone accomplishes little; the treatment with hot alcohol-ether, however, removes most of the lipids. Following the work of McFarlane on serum lipids, experiments were carried out on the effect of ether extraction of the thromboplastic protein in the frozen state which gave interesting results.<sup>13, 14</sup>

<sup>7</sup> Chargaff, *J. Biol. Chem.*, 1945, **161**, 389.

<sup>8</sup> Chargaff and West, *ibid.*, 1946, **166**, 189.

<sup>9</sup> Chargaff, *ibid.*, 1948, **173**, 253.

<sup>10</sup> Chargaff and Green, *ibid.*, 1948, **173**, 263.

<sup>11</sup> Cohen and Chargaff, *ibid.*, 1940, **136**, 243.

<sup>12</sup> Chargaff, Moore and Bendich, *ibid.*, 1942, **145**, 593.

<sup>13</sup> Chargaff and Bendich, *Science*, 1944, **90**, 147.

<sup>14</sup> Chargaff, Bendich and Cohen, *J. Biol. Chem.*, 1944, **156**, 161.

It is not certain that a single conception of the structure of tissue lipo-proteins will be applicable to the various compounds encountered. It would seem that the thromboplastic protein occurs in the form of extremely heavy particles composed of alternating lipid and protein layers. These particles may be assumed to be surrounded by a water barrier which prevents cleavage by ether alone, but can be removed by freezing.

As to the localization of the thromboplastic protein within the cell, it could be shown that the activity was confined to one cell fraction; the nuclei and the soluble proteins of the cytoplasm were free of activity.<sup>15</sup> The cell membrane could not be investigated.

Many other types of cellular lipo-proteins could be discussed; but this would not be very helpful since our ignorance of their structure and behaviour is almost complete. In conclusion, some work, recently carried out in our laboratory, on the macromolecular lipo-proteins of plants and of yeast will be mentioned.

The chromoproteins of animal and plant cells represent important instances of lipid-protein complexes. One could mention the very labile

TABLE I.—COMPOSITION OF CHROMOPROTEINS

Source	Sedimentation in centrifuge	Composition of lipo-protein			Composition of lipid-free residue	
		N	P	Total lipids	N	P
		%	%	%	%	%
Spinach . .	5,000 g	9.7	0.12	32.4	13.8	0.11
	31,000 g	9.4	0.19	32.0	13.2	0.16
Carrot . .	5,000 g	5.6	1.3	23.4	7.7	1.3
	31,000 g	6.4	1.1	31.8	8.0	0.7

visual purple and also the chloroplasts and chromoplasts of plants. The occurrence of coloured macromolecules in plant juices has been known for a considerable time. Recent work on the green lipo-protein particles of spinach leaves and the orange-coloured particles of carrots was carried out, in collaboration with Dr. M. A. Nyman, with the purpose of using the same centrifugal methods that had been applied to the study of the thromboplastic protein from animal tissue. The composition of some coloured lipo-proteins of plants is shown in Table I. The spectrum of the spinach macromolecule shows maxima at 4400 and 6820 Å. The yields of this green protein vary somewhat, but lie around 1 g. per kilogram of spinach leaves. It was of interest to determine whether the pigment distribution in these isolated fractions differed from that of the total leaves. This does not seem to be the case. The pigments attached to these complexes could be separated by chromatographic adsorption and shown to consist of chlorophyll *a* and *b*, of carotenoids (mostly  $\beta$  carotene with about 10 %  $\alpha$ ), and of xanthophylls. The protein residue, following the removal of lipids and pigments, was low in phosphorus. Occasionally preparations were, however, obtained with a higher non-lipid P; and the important

<sup>15</sup> Chargaff, *J. Biol. Chem.*, 1945, **160**, 351.



question whether the lipo-protein granules of the chloroplasts are nucleoprotein complexes will have to await a more conclusive answer.

The spectrum of the orange-coloured preparation from carrot showed a maximum at 2700 Å and a plateau with several small peaks between 4200 and 5000 Å. The small particles, sedimented at very high centrifugal speed, and the coarse particles, deposited at about 5000 g, both had very similar spectra. The yields were 0.11 to 0.17 g. per kilogram of carrots. The lipid-free protein residue was quite rich in phosphorus, some of which appeared to be pentose nucleic acid, but the bulk a phosphoprotein.

Recently, an orienting study of the lipo-proteins from yeast cells was carried out.<sup>16</sup> When washed yeast cells are ground in a bacterial mill, the supernatant, following centrifugation at 1900 g, is opalescent and deposits a sediment at 31,000 g which proves to be almost homogeneous in the electrophoresis cell. The lipids (22 to 26 % of the material) were characterized by an extremely high iodine value (about 130) and contained roughly 20 % ergosterol. The study of the phosphorus distribution in the lipid-free protein residue revealed about 12 % acid-soluble P, 26 % lipid P, 35 % nucleic acid P (of which about one-seventh consisted of desoxyribonucleic acid), 12 % "phosphoprotein."

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<sup>16</sup> Nyman and Chargaff, *J. Biol. Chem.* (in press).

### GENERAL DISCUSSION \*

**Prof. E. J. Cohn** (*Harvard*) said: I was delighted to hear Claude's magnificent contribution. The return to the study of morphology with chemical methods brings to mind the courageous departure, early in this century, of W. B. Hardy, from traditional cytology in quest of the chemical nature of the interactions with native proteins of reagents then in the "microtomists vade maecum." Although Sir William did not himself return to the study of morphology, his studies laid the foundation not only for a new colloid chemistry but for a new chemical morphology.

During the last three years, a large group of able investigators have collaborated † to develop a satisfactory system for the chemical fractionation of liver. When precautions are taken to permit separation of the components of liver more nearly in their state in nature, vitamins, such as the active principle effective in pernicious anaemia,<sup>1</sup> and enzymes, such as the catalase crystallized by Sumner, have been found to exist

\* On preceding paper and the lecture delivered by Prof. A. Claude (Brussels).

† Including B. Norberg from Sweden, 1946-47; Derouaux from Belgium, 1947-48; H. Nitschmann from Switzerland, 1947-48; M. J. Hunter from Scotland, 1948; R. W. Greene, 1948-49; F. W. Kahnt from Switzerland, 1948-49; and D. M. Surgenor in our laboratory; J. D. Porsche and J. B. Lesh in the Armour Laboratories; and a Committee of the Haematology Study Section, National Institute of Health.

<sup>1</sup> E. J. Cohn, D. M. Surgenor, R. W. Greene, J. D. Porsche, J. B. Lesh, and a Committee of the Haematology Study Section, National Institutes of Health, and others including B. Norberg, Sweden; G. Derouaux, Belgium; and H. Nitschmann, Switzerland, *The Preservation of the Formed Elements and of the Proteins of the Blood*, American Red Cross, 1949, p. 257.

as parts of large protein complexes.<sup>2</sup> In our new process, in the interest of maintaining components in the solid state fractional extraction in ethanol-water mixtures is employed, rather than gross extraction followed by fractional precipitation. Furthermore the system is designed to extract enzymes in fractions which do not contain their substrates. Thus esterase, phosphatase and xanthine oxidase are initially extracted in one direction before separation by sub-fractionation, catalase complex and the protein bearing the active principle effective in pernicious anaemia in another, cathepsins, desoxypentosenucleoprotein, and pentosenucleoprotein in still others.

The possibility of employing the general principles of our ethanol-water fractionation system to discover the distribution of the proteins in a tissue was considered with Prof. Caspersson of Stockholm on the occasion of his Dunham Lectures at Harvard in 1946. Where, in our large-scale operations comminution in the frozen state is employed to break cell walls, fractional extraction from frozen sections cut by the microtome, was envisaged. The temperature in the frozen section, as in the chemical process, is raised to just above the freezing point for the purposes of the extraction. After each extraction the section would immediately be returned to the frozen state.

Using chemical methods it was found, by Norberg, that no protein was extracted at low ionic strength near pH 6 by 60 % ethanol (by volume at 25° C) at - 30°. A considerable number of the most soluble proteins have now been identified in the fraction extracted at 35 % ethanol at - 18°; still others at lower ethanol concentrations and higher temperatures, all below 0° C. However, the nucleoproteins remain in the solid state, resisting extraction at low ionic strength, at this pH, in the presence of even small amounts of ethanol. They are, however, readily dissolved and purified after all proteins and any protein enzymes that are soluble in 10 % ethanol at - 3° have been separated. When lipo-proteins are to be extracted in their native state, freezing should clearly be avoided. No cholesterol has, however, been found in the desoxypentosenucleoprotein, purified by our present procedures.

In reply to Prof. Claude, unfortunately, or fortunately, we are working with 50 kg. batches of fresh bovine livers, perfused so as to remove blood proteins, chill and inhibit enzyme action as rapidly as our present technique permits. We have feared the delays of introducing mechanical separations before bringing the system to a more stable state by enzyme inactivation or separation. Further technical developments which are planned should make it possible more nearly to approximate the state in nature of the protein components separated from a tissue.

**Prof. A. Frey-Wyssling (Zürich)** said: Whether the membranes of cells, chloroplasts, mitochondria, nuclei and nucleoli are more lipid membranes or lipo-proteins can be decided by cytological observations. If such a membrane shows active changes of shape (amoeboid movement) as is known from cell membranes and from chloroplast membranes, there must be some contractile protein involved.

**Prof. A. Claude (Brussels)** said: Electron-micrographs of cell membranes, especially those of haemolyzed erythrocytes, and of the membrane of cell inclusions such as nuclei, mitochondria, and chloroplasts, indicate that membranes are differentiated structures, distinct from the underlying cytoplasm. It is possible that proteins take part in the constitution of membranes of living cells. The existence of amoeboid movements does not imply that contracting proteins must be present. The plastic properties of phospholipid membranes is well known, and myelin figures can duplicate many aspects of the morphology and movements of living

<sup>2</sup> E. J. Cohn, D. M. Surgenor, R. W. Greene, M. J. Hunter, F. W. Kahnt, and others including B. Norberg, Sweden, in 1946-47; G. Derouaux, Belgium, and H. Nitschmann, Switzerland, in 1947-48, *Science*, 1949, **109**, 443.

cells. In fact, the presence of proteins seem to restrain the formation and mobilization of myelin figures.<sup>3</sup>

**Dr. A. B. L. Beznak** (*Birmingham*) said: The investigations of Prof. Claude, as also other recent researches, prove the particulate structure of the cell interior. The question thus arises: how are the functions of these specific units co-ordinated and integrated into the life processes of one organized cell?

Hopkins<sup>4</sup> laid out the details and emphasized the importance of the specificity of the molecular structure of certain catalysts within the cell. In the chemical organization diffusion is the vehicle taking the organizing molecules from one spot to another and the specificity of the organizing molecule is the factor determining which of the functioning units will be activated or inhibited, i.e. organized.

R. A. Peters spoke of a cytoskeleton protein acting also as an impulse conductor between the cell membrane and the nucleus. Histological methods by staining with the impregnation of neuro-fibrils produce both in unicellular organisms (*Geleia paramaecium*) as well as in the cells of multicellular organisms pictures of a fibrillar system (*Huzella*).

Prof. Claude suggested the function of the lipids to be the forming of a structural network—cytoskeleton—in the cell. What is his opinion about the value of the histological observations as proofs of the existence of a conducting fibril system in the cell? Are these—as is in his view the Golgi apparatus—artefacts, or does the living cell possess—similarly to the multicellular organism—both chemical organization and a kind of nervous conducting system?

**Prof. A. Claude** (*Brussels*) said: Investigations of recent years have demonstrated that a cell is not a continuum but is essentially composite, made up of entities that are distinct in organization and chemical composition and, consequently, in functions. The co-ordination and integration of the specialized activities of these various components: chromosomes, nucleoli, mitochondria, centrioles and, possibly, microsomes, find its expression in the life of the cell. The question raised by Dr. Beznak is two-fold: (a) how are the biochemical processes performed within each morphological cell constituent and (b) how is the overall activities of the separate cell constituents mediated and co-ordinated within the cell.

Mitochondria, for example, are small elements limited by a semi-permeable membrane which, like that of the cell, is probably endowed with selective properties. Within the boundary of this membrane are enclosed various enzymatic systems such as the cytochrome-linked system, the fatty acid oxidase system, etc. It is possible that substrates (amino acids, etc.) are brought to the cytoplasmic granules by diffusion, and that certain end-products are carried away by the same process; in view of the high specificity of biological reactions, however, and the fact that some of the enzymes mentioned have been found to be bound to phospholipid-protein complexes, it would seem more probable that important parts of the metabolic activity of a cell, involving definite sequences of chemical reactions, are performed through the agency of rigid structures located, in the case considered, in the body of cytoplasmic granules.

Interposed between the various cytoplasmic granules is the so-called ground substance, in which two major components have been recognized: (a) the microsome substance (15 % or more of the cell mass) and (b) an abundant, fibrous protein which is demonstrated especially by acid fixatives, and which is supposed to give to the cytoplasm its plastic properties. It is possible that the spindle and asters, organized in the cytoplasm at the time of mitotic division, belong to the same fibrous protein system. Observations on living cells indicate that this protein component of the ground substance is not a fixed structure but that,

<sup>3</sup> Palade and Claude, *J. Morphology*, 1949, **85**, 35 and 71.

<sup>4</sup> *Second Purser Memorial Lecture* (1932).

through gel and sol formation, it contributes to the motility of the cell and to cytoplasmic streaming. The microsomes may be caught in this fibrous network, or even take part in the phenomenon of reversible gelation, but there is no information on this point.

Lipids, especially phospholipids, have been found to constitute an appreciable proportion of the mass of mitochondria (about 25 %) and microsomes, where they occur in firm association with proteins and nucleic acids: the assumption is that phospholipids, through their hydrophobic-hydrophilic properties may contribute to the formation of stable structures by restraining the diffusion of otherwise soluble proteins. If these proteins are enzymes, as in mitochondria, it is conceivable that the presence of phospholipids may help in the constitution of reactive surfaces, and in the regulation of exchanges with the aqueous medium. Some of these points have been discussed in greater details in two preceding papers.<sup>5</sup>

**Prof. T. R. Parsons** (*Nigeria*) said: Seeing that the mitochondria are the carriers of important enzyme systems and are in constant movement in the cell protoplasm, is it possible that this movement is directed in such a way as to carry the enzyme system to the substrate on which it is to act, and so to assist in the organization of chemical events in the cell?

**Prof. A. Claude** (*Brussels*) said: Movements of mitochondria, and cytoplasmic streaming, must activate exchanges, and insure uniformity in the medium.

**Dr. A. Lasnitzki** (*Birmingham*) said: I should like to ask Prof. Claude: (1) whether there are conclusive results which indicate that mitochondria are endowed with the property of self-division, especially during the process of cell proliferation, and if so, (2) whether there is any evidence that the self-division of each mitochondrion is accompanied by a co-ordinated self-division of each of the various enzymes contained in it. I think it is improbable that the regeneration of enzymes during cell proliferation takes place through a synthesis of new enzyme molecules from the available building material (amino acids, prosthetic groups, etc.), since this synthesis would require the presence of other enzymes which might be called "superenzymes". Obviously, these would also have to undergo regeneration in the course of that process and thus the question of origin would merely be shifted from the enzyme to the super-enzyme. For this reason the self-division of enzymes becomes the far more probable alternative, and it can well be said that the problems involved may open up, at some future time, a new and promising field of enzyme research. Further, it would be interesting to know: (3) whether in the tiny body of a mitochondrion, with its considerable enzymatic activity, there will be much room for any kind of protein which is not enzyme-protein, and I may mention in this connection that some time ago Virtanen<sup>6</sup> has come to the conclusion that, at any rate, the main portion of protein contained, for example, in a yeast cell is likely to consist of enzyme-protein.

**Prof. A. Claude** (*Brussels*) said: In contrast to chromosomes, which duplicate according to their length, mitochondria appear to grow by elongation, and may fragment during cell activity. In certain germ cells, mitochondria become arranged in ring or bundles, perpendicular to the metaphase plate, and are divided passively by the constricting furrow, so that opposite halves are retained by the two daughter cells. It is clear that enzymes or complexes of enzymes must be uniformly distributed along the filament; otherwise, repeated, transverse sub-division of a mitochondrion would eventually result in segregation and in loss of functions.

In recent years, attention has been centred on the problem of

<sup>5</sup> *The Harvey Lectures*, 1947-48, **43**, 121; *Advances in Protein Chemistry*, 1949, **5**, 423.

<sup>6</sup> Virtanen, *Suomen Kemistilehti B*, 1942, **15**.

re-duplication of gene substance, although it is obvious that all the other cell structures are also being reproduced during cell growth, or at the time of cell division. It is probably an error to attempt to visualize reduplication as a unique event, dissociated from other, concomitant activities of the cell. The activity of a cell is the expression of the integration of individual and diverse, but tightly interlocked, biochemical activities. When speaking of self-duplication it should be remembered that the synthesis of a single protein molecule must involve many specific enzymes, working in proper sequence, and an adequate supply of energy. The reduplication of a part of a gene, on the one hand, the production of a particular enzyme in a mitochondrion, on the other hand, may be but intermediate steps in a single, however complex, biochemical cycle, each product in turn taking part and thereby directing the speed and specificity of the same, or other interlocked chemical cycles.

It has been calculated <sup>7</sup> that the solid matter present in a mitochondrion, 2  $\mu$  long and 0.5  $\mu$  diam., would amount to  $1.4 \times 10^{-7}$  g., and that proteins may account for about one-half of the mitochondrial body, or about  $0.7 \times 10^{-7}$  g. of the bulk. If we assume that each mitochondrion has a complement of, let us say, 25 different enzymatic systems, each such system being composed of 20 different protein molecules, it appears that there could exist, simultaneously in the same mitochondrial unit, as many as 2000 duplicates of each of the 25 enzymes postulated. If we remember that only a few enzymes have been detected in mitochondria, there is room for many more to be isolated or discovered.

**Dr. G. Popjak** (London) said: Prof. Claude has asked for any information that might be available regarding the nature of function of microsomes. Gordon Ada, from Australia, working at the National Institute for Medical Research, studied the turnover of phospholipids with the aid of  $P^{32}$  in the "mitochondria," "microsomes," and "supernate" prepared by differential centrifugation from rabbit liver. The specific activities of phospholipid-P (counts/min. mg. P) were followed from  $\frac{1}{2}$  to 72 hr. after the injection of inorganic  $32PO_4^{3-}$ . Three distinct specific activity-time curves were obtained: at all time intervals the specific activity of the microsome phospholipids was the highest, next in order being the phospholipids obtained from mitochondria. The phospholipids extracted from the supernate had the lowest specific activities. Since these three curves never crossed each other, Ada concluded—quite safely, I think—that within the intact cell the microsomes have a metabolism distinct from that of mitochondria and that the microsomes are not derived from mitochondria. The relationship between the specific activity time curves of mitochondria—and plasma-phospholipids was such as to suggest strongly that the latter are derived from the former according to the criteria of Zilversmit, Entenman and Fishler.

**Prof. A. Claude** (Brussels) said: The demonstration of the microsomes as particulate constituents of cytoplasm rests mainly on their isolation by differential centrifugation. At the moment, the evidence derived from electron microscopy is not entirely conclusive; on the other hand, attempts to demonstrate in microsomes distinctive biochemical properties have not been successful. The position that could be taken, with respect to the nature of the microsomes, may be (a) that the microsomes exist as distinct particulate elements in living cytoplasm, or (b) that they derive from the breakdown, during tissue manipulations, of other cell constituents. For instance, the microsomes might be suspected to represent debris of mitochondria, since both have been found to contain the same types of lipids, and ribose nucleic acid. The introduction by Palade <sup>8</sup> of hypertonic sucrose as a medium of choice for the isolation of mitochondria led to results which seem to support the view that mitochondria and microsomes are separate entities: in sucrose, mitochondria

<sup>7</sup> Claude, *Advances in Protein Chemistry*, 1949, **5**, 423.

<sup>8</sup> *Proc. Soc. Exptl. Biol. Med.*, 1947, **65**, 320.

appear exceptionally well preserved and they retain for a considerable time their specific activity; microsomes separated from sucrose extracts appear also more stable, and no less abundant than when obtained from saline extracts.

The data mentioned by Dr. Popjak are interesting in that, to my knowledge, they constitute the first results pointing to a fundamental difference in the origin of microsomes and mitochondria. The use of isotopes should furnish results of the same interest regarding the origin of ribose nucleic acid in the same cytoplasmic elements.

**Prof. A. C. Frazer** (*Birmingham*) said: Prof. Claude's remarks on the Golgi apparatus are most interesting. The Golgi apparatus is well developed in the cell of the small intestine and it increases markedly in size during fat absorption. Histochemically the Golgi apparatus gives the usual reactions of phospholipid. Phospholipid is probably formed in increased quantities during fat absorption provided that the cell is not deprived of the essential raw materials for phospholipid formation. At the same time there are undoubtedly streaming effects within the cell due to the absorption of water and other materials. Thus the conditions described by Prof. Claude are fulfilled. However, even if the Golgi apparatus is eventually shown to have little morphological validity in living cells, its existence or development under standard conditions may still provide useful information with regard to intracellular activity.

**Prof. A. Claude** (*Brussels*) said: The data presented in the recent papers of Palade and Claude<sup>\*</sup> demonstrate that the Golgi apparatus is represented by myelin figures produced in cells during fixation and impregnation. These observations do not detract from the fact that different cells may have distinctive lipid metabolisms, and that the distribution in cells of free phospholipid droplets, which give rise to myelin figures, may be indicative of typical cell activity, for example, intensive phospholipid metabolism, or oriented cytoplasmic streaming.

**Dr. A. S. McFarlane** (*London*) said: I would like to draw attention to a letter in *Nature* recently, contending that the chromatinic threads prepared by Claude's and other processes bear no morphological relationship to chromosomes. I have been engaged in studies of fragments of avian erythrocyte nuclei and I can subscribe to the view that the size and shape of the chromatinic threads depends on the shearing force used to break the nucleus and the manner of its application. In drawing attention to this, I do not wish to detract from the merits of Dr. Claude's general technique of separation, which, so far as other cell structures are concerned, I find to be most reliable.

**Prof. A. Claude** (*Brussels*) said: In our original paper with Potter, we draw the attention to the evident relation between the strands that we had isolated from resting nuclei, and chromosomes, but we were careful to refer to them mainly as chromatin threads: cytologists agree, I believe, that metaphase chromosomes differ morphologically from the chromatin formations seen in "resting" nuclei.

I concur with Dr. Macfarlane that chromatin threads may be profoundly affected by the shearing action, and the traction, to which they are subjected during mechanical separation. However, our original data, as well as recent observations on sedimented nuclei and especially on phase difference microscopy of living cells in tissue culture, indicate that resting nuclei actually contain chromatin threads resembling those isolated by differential centrifugation.

The conclusion based on the electron micrograph published in the paper of Lamb (referred to in this Discussion) is obviously unwarranted: the preparation used for electron microscopy was too thick to permit differential absorption and transmission of electrons and, consequently, could not reveal details of structure. In the electron microscope, salivary

<sup>\*</sup> *J. Morphology*, 1949, **85**, 35, 71.

glands chromosomes of *Drosophila* are uniformly absorbing; replica of the same elements reveal the fine morphology already demonstrated by light microscopy.<sup>10</sup>

There is no doubt that the chromatin isolated by differential centrifugation is altered not only morphologically, but also chemically. The physiological saline used is a precipitant of desoxyribonucleoproteins and, therefore, may not be a favourable medium for the preservation of the morphology of chromatin.

<sup>10</sup> Palay and Claude, *J. Expt. Med.*, 1949, **89**, 431.

## MORPHOLOGICAL ASPECTS OF THE LIPO-PROTEINS IN CHLOROPLASTS

BY A. FREY-WYSSLING

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The chloroplast of higher plants consists of (a) a membrane of fibrillar proteins; (b) a stroma of globular proteins (no gel-like framework has been discovered as yet, although one might exist in the living chloroplast); (c) granules, with possibly a layered protein texture. Probably all three elements contain lipo-proteins when in the intact chloroplast, but as soon as the chloroplast is isolated and prepared for closer morphological examination, the lipids dissociate from the protein as myelin forms. Therefore, we do not know the exact location of the lipids in the membrane and the stroma, but in the granules they probably are adsorbed as molecular films.

The biochemical investigation of the chloroplast destroys these details of the structure, and what is actually examined is a mixture of at least three morphologically different things. A real understanding of the chemical components of chloroplasts will be possible only after methods have been developed to separate these tiny elements from each other and to analyze them individually.

Chloroplasts have a very high content of extractable lipids. Precipitated chloroplast material from spinach leaves, as well as whole chloroplasts separated by centrifuging have the composition<sup>1</sup> shown in Table I. The ratio lipids/protein is about 2/3, or, with isolated

TABLE I.—ANALYSIS OF CHLOROPLASTIC MATTER OF *Spinacia oleracea* IN % BY WEIGHT

	Menke <sup>2</sup>	Chibnall <sup>3</sup>	Bot <sup>4(a)</sup>	Comar <sup>4(b)</sup>
Lipids . . .	37.4	25.1	26-32	34
Protein . . .	47.7	39.6	42-54	54
Ash . . .	7.8	16.9	—	7
Residue . . .	7.1	18.4	16-25	—

<sup>1</sup> Rabinowitch, *Photosynthesis and Related Processes* (Interscience, New York, 1945), Vol. I, p. 372.

<sup>2</sup> Menke, *Z. physiol. Chem.*, 1938, **257**, 44.

<sup>3</sup> Chibnall, *Protein Metabolism in the Plant* (Yale Univ. Press, New Haven and Oxford Univ. Press, London, 1939), p. 137.

<sup>4</sup> (a) Bot, *Chronica Bot.*, 1942, **7**, 66, and *Diss.* (Leiden, 1939). (b) Comar, *Bot. Gaz.*, 1942, **104**, 122.

chloroplasts (Menke<sup>2</sup>), even  $3/4$ . Half of the lipids consist of fats, 20 % of sterines, 16 % of raw wax, and 2-7 % of phosphatides (Menke and Jacob<sup>5</sup>).

**Results of Light Microscopy.**—The morphological distribution of these lipids is peculiar. The chloroplasts of higher plants have a granulated structure, with green granules imbedded in a colourless stroma. These granules have a diameter of a fraction of a  $\mu$ ; they are not globular but rather have the shape of small discs. Staining experiments with Sudan or Rhodamin B yield an accumulation of these fat-staining dyes in the granules, and leave the stroma unstained (Weier,<sup>6</sup> Strugger<sup>7</sup>). This would indicate that the lipids are located in the granules. These granules, however, do not consist merely of lipids and pigments. If the chloroplasts are treated with fat solvents, the lipids are extracted, but the granules remain (Granick,<sup>8</sup> Menke<sup>9</sup>), so they must contain a protein ground mass. This is also indicated by their shape, which ought to be spherical if there were no structural proteins present.

On the other hand, it is not possible that all the lipids of the chloroplast are located within the granules, because their total mass is much too small to contain  $1/4$  or even  $1/3$  of the dry weight of the chloroplast which the lipids make up (see Table I). According to Granick,<sup>8</sup> the chloroplast of spinach contains 40-60 granules,  $0.6 \mu$  in diameter and  $0.08 \mu$  thick. Since in some instances it has been possible to photograph the granules in profile with the light microscope (Heitz<sup>10</sup>), this submicroscopic thickness of  $0.08 \mu$  may be due to desiccation during the preparation for the electron microscope, and we may estimate the thickness of the fresh granules to be about  $0.15 \mu$ . The whole chloroplast has a diameter of  $5 \mu$  and its thickness in the fresh state is about half of this. If we calculate the volume of the chloroplast as an ellipsoid,  $4/3 \times 2.5^2 \times 1.25 \times \pi$ , and that of the 50 granules as cylindrical discs,  $50 \times 0.3^2 \times 0.15 \times \pi$ , we obtain a volume ratio of 15/1. Thus, the total volume of the granules is only  $1/15$  that of the whole chloroplast, obviously not enough to hold all the lipids of the chloroplast, especially if we bear in mind that the granules contain proteins in addition.

We must conclude, therefore, that the stroma is also rich in lipids, although these lipids show no staining reaction. Both granules and stroma contain proteins, and we may ask what kind of interrelation exists between lipids and proteins in these different parts of the chloroplast. If we assume a close connection to form a lipo-protein within the stroma, it may be that the protein which carries the lipid molecules is so hydrophilic and the lipid molecules so far apart from each other that fat-staining dyes cannot be accumulated. If such a view is to be reasonable, one must assume the arrangement of the lipid molecules in the granules to be such that dye molecules can be stored in between them. This would mean a closer packing of the lipid chains, e.g. by forming submicroscopic regions of pure lipid matter alternating with regions of protein. In this way another type of lipo-protein

<sup>2</sup> Menke and Jacob, *Z. physiol. Chem.*, 1942, **272**, 227.

<sup>6</sup> Weier, *Amer. J. Bot.*, 1936, **23**, 645.

<sup>7</sup> Strugger, *Flora*, 1937, **31**, 324.

<sup>8</sup> Granick, in Franck and Loomis, *Photosynthesis in Plants* (Iowa State College Press, Ames, Iowa, 1949), p. 113.

<sup>5</sup> Menke, *Protoplasma*, 1940, **35**, 115.

<sup>10</sup> Heitz, *Planta*, 1932, **18**, 616.



would occur, which, depending on the size of those regions, would represent a colloidal mixture rather than a chemical compound. Probably the bonds between protein and lipids are looser in the granules than in the stroma.

Morphological studies allow of characterizing the type of bonds in the stroma. When chloroplasts are treated with ammonia or other dilute alkalis, the lipids leave the chloroplasts and produce myelin tubes (Weber,<sup>11</sup> Menke<sup>12</sup>). This dissociation of protein and lipids seems to be a saponification. As it is also provoked by alkaline salts such as Na carbonate, Na oleate, Na glycinate, or even urea, which induce only a very mild saponification, the ester bond between lipids and protein must be very weak.

We must conclude that not only the stroma lipids, but also those in the granules, are affected by this treatment with bases, because the chlorophyll, which is located in the granules, is removed by the treatment and stains the myelin forms green.

**Results of Electron Microscopy.**—The cytological facts reported here will be used to interpret the electron micrographs of chloroplasts which have already been published.<sup>8, 9, 13 17</sup> Besides stroma and granules, a distinct plastid membrane has been disclosed<sup>17</sup> as a third morphological element of the chloroplast. This membrane must consist essentially of proteins, as it displays the properties of a solid veil and does not show any sign of the liquid or semi-liquid state characteristic of lipid matter. It is probable that the living membrane contains lipids, but their amount must be small as compared with the total lipid mass in the chloroplast. Obviously they join the emigrating myelin. The proteins of this plastid skin must be of the fibrous type; otherwise the formation of a textured membrane would not be possible.

Under the membrane, the granules are visible as massy discs. The stroma, on the other hand, does not show a conspicuous structure. A beautiful photograph presented by Wyckoff<sup>18</sup> gives evidence of globular macromolecules about 250-300 Å in diameter, which lie on and between the granules. If the plastid membrane has burst, as usually occurs during the preparation of the chloroplasts, the whole carrier film is sprinkled with these globular bodies. This behaviour would indicate that the stroma is a corpuscular dispersion of macromolecules, i.e. a sol. Since a sol has no framework, the characteristic shape of the chloroplast must be due to its membrane, much the same as in erythrocytes. This notwithstanding, there must be some organization within the stroma, but this problem cannot be attacked as yet. The chloroplast can change its shape,<sup>18</sup> or even form processes;<sup>10</sup> this faculty must be ascribed to the membrane, which may be compared with the ectoplasm of creeping protobionts. This again argues rather for a protein than a lipid ground mass of the plastid membrane.

<sup>11</sup> Weber, *Protoplasma*, 1933, **19**, 455.

<sup>12</sup> Menke, *ibid.*, 1934, **21**, 279.

<sup>13</sup> Kausche und Ruska, *Naturwiss.*, 1940, **28**, 303.

<sup>14</sup> Algera, Beyer, v. Iterson, Karstens and Thung, *Biochim. Biophys. Acta*, 1947, **1**, 517.

<sup>15</sup> Granick and Porter, *Amer. J. Bot.*, 1947, **34**, 545.

<sup>16</sup> Wyckoff (in press).

<sup>17</sup> Frey-Wyssling and Mühlethaler, *Viertelj. schr. Naturf. Ges. Zürich*, 1949, **94** (in press).

<sup>18</sup> Senn, *Die Gestalts- und Lageveränderungen der Pflanzen-Chromatophoren*. (Engelmann, Leipzig, 1908).

We may ask whether the macromolecules found by Wyckoff represent lipo-proteins or only proteins. It is almost certain that the latter is the case. The preparations show very thin flat discs<sup>8, 9, 14, 17</sup> of various diameters up to  $5\mu$  and only 100-200 Å thick. It can be shown that before desiccation these discs were in a semi-liquid state. They never have folds, as the plastid membrane does, and dry perfectly smooth on the carrier film, even if they include isolated granules. There has been much discussion on the nature of these discs. They have been looked upon as protein lamellae<sup>9</sup> or phosphatide bladders<sup>14</sup> (which is unlikely, as the chloroplast contains only 0.5-2.5 % phosphatides), but there is no doubt that they represent the total lipid matter of the chloroplast and must be considered as myelin forms. Thus, during preparation and drying of the chloroplast, the lipo-protein of the stroma disintegrates into myelinic matter which dries as smooth discs and globular protein macromolecules.

It is likely that the lipids of the granules have also emigrated, because the granules as seen in the electron microscope consist merely of proteins. Washing with lipo-solvents does not alter them.<sup>9, 15</sup> They seem to be layered much like a low pile of coins. Occasionally such a pile appears to be overturned;<sup>17</sup> then a series of very thin lamellae, all of the same diameter, are visible. These must consist of protein. In the living state, the lipids in the granules probably were located between these protein layers. If this picture can be substantiated by further research, the granules of the chloroplast would represent a layered composite body with alternating protein and lipid lamellae. The chlorophyll is in close relationship with the granule lipids, because it emigrates together with them; on the other hand, this pigment must be combined with the proteins for the transmission of the captured light energy. It will be an interesting problem to find out how the chemical notion of a lipochromoprotein fits in with the morphological conception of a layer composite body.

**Results of Polarization Microscopy.**—There is a method of proving the existence of a layered body with the aid of polarized light and a special imbibition technique. As the granules are too small to be investigated in the polarizing microscope, the big chloroplasts of the alga *Mougeotia* have been used for this study.<sup>19</sup> They have the shape of a rather thick plate which is as long and wide as the whole cell in which it is located. Further, they appear homogeneous in the light microscope, no granules having been detected as yet. When such a chloroplast is fixed and then observed in mixtures of acetone and methylene iodide with refractive indices  $n$  increasing from 1.36 to 1.74, the double refraction changes following a hyperbolic curve. According to Wiener's theory of the anisotropy of composite bodies, this behaviour discloses a layered body, the lamellae of which are thin compared with the wave lengths of light. When the imbibition is made with a mixture of  $n = 1.58$ , the chloroplast becomes isotropic. This is the point where the lamellae have the same refractive index as the imbibing medium. As acetone removes the lipids the disclosed lamellae must consist of protein. It is of interest to note that muscle protein and neurokeratin from nerve sheaths<sup>20</sup> also have a refractive power as high as 1.58.

<sup>19</sup> Frey-Wyssling and Steinmann, *Biochim. Biophys. Acta*, 1948, **2**, 254.

<sup>20</sup> Schmidt, *Z. wiss. Mikroskopie*, 1937, **54**, 159.

If the chloroplasts are fixed with  $\text{OsO}_4$ , the lipids become partly insoluble. Then we find, in addition to the variable lamellar double refraction above, a constant intrinsic anisotropy, independent of the refractive power of the imbibition medium, which is due to orientated adsorbed lipids.

Thus, the chloroplast of *Mougeotia* is a microscopic object with a submicroscopic layered texture of protein lamellae and interposed orientated lipid matter. As related above there is some indication that the granules of the chloroplasts of higher plants have a similar texture; and it would be of importance for the understanding of assimilation if the principle of lamination could be definitely established as a general principle for all chloroplasts.

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## THE EFFECT OF DRYING UPON THE STRUCTURE OF MYELIN IN THE SCIATIC NERVE OF THE FROG

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Using suitable observation cells, X-ray diffraction studies of frog nerve myelin have been carried out. The effect of slow drying and of lipid solvents upon this lipo-protein has been studied. Three stages could be distinguished in the drying process. Of these, the changes noted in stages I and II were reversible; those seen in stage III were irreversible. Differences in the susceptibility of various spacings to lipid solvents were also observed. The diffraction spacings are given and the possible structure of the myelin unit is discussed.

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Preliminary observations on the structure of frog nerve myelin have already been reported,<sup>3</sup> and served to confirm several findings of previous authors.<sup>1, 2</sup> The present experiments are essentially concerned with the effect of drying upon this lipo-protein.

### Experimental

**Source of Radiation and Diffraction Cameras.**—Nickel filtered copper  $K_\alpha$  radiation from a 5 kW rotating anode X-ray tube was used wherever possible. In experiments involving serial observation, short exposure was essential, and the radiation used here was not filtered. The diffraction cameras have been previously described.<sup>3</sup> In most of the experiments it was inconvenient to work *in vacuo*. A glass tube, of varying length and about 3 in. diam., sealed at either end with cellophane, was therefore interposed between specimen and film. When filled with hydrogen this effectively reduced scatter by air.

<sup>1</sup> Schmitt, Bear and Clark, *Radiology*, 1935, **25**, 131.

<sup>2</sup> Schmitt, Bear and Palmer, *J. Cell. Comp. Physiol.*, 1941, **18**, 31.

<sup>3</sup> Elkes and Finean, 1949 (in press).

**Observation Cells.**—Only two are described. A number of modifications were used in initial experiments. The first of these (Fig. 1 and 2) aimed at providing a means of exposing a section of frog sciatic nerve to varying degrees of humidity and to the vapours of a number of solvents, while at the same time ensuring adequate wetting throughout the rest of the preparation, and recording variations of X-ray diffraction patterns in the affected segment. Provision for recording the action potential was also made. The cell was constructed of polythene. It consisted essentially of four compartments: an irrigation cap A containing (according to the type of experiment) the spinal column or gastrocnemius muscle; compartment B, through which the moist nerve passed into vapour chamber C. This was fitted with inlet and outlet tubes, making possible

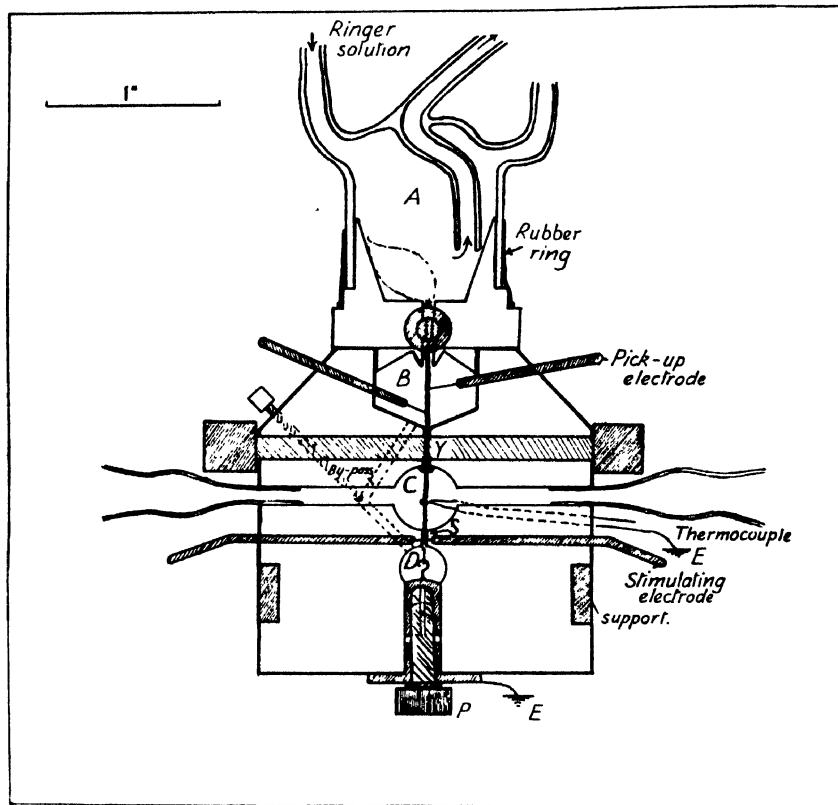


FIG. 1.—Diagram of irrigation cell.

the passage of vapours through the chamber at controlled rates and temperatures. Cellophane windows were provided for the passage of X-rays. The bottom chamber D was connected to chamber C by a short capillary S, through which the nerve was passed before being attached to a small hook in chamber D. The position of this hook could be varied so as to bring the nerve under slight tension. Frog Ringer solution was circulated continuously through irrigation cap A. Its slow drip down the nerve through compartment B, was regulated by valve X. A further valve Y was used to regulate the passage of Ringer solution along the nerve through compartment C. Compartment D was always filled with Ringer solution, any excess overflowing by way of an overflow tube. The section of the nerve in capillary S was thus always kept moist by a

short column of Ringer solution. A by-pass, short-circuiting B into D, was also provided. With valve Y closed, this lead the Ringer solution past compartment C so that the environment of the nerve segment in C could be altered independently of its condition in capillary S and compartment B. A thermocouple was kept in contact with the nerve in compartment C, and also served as the earthing electrode in the action potential circuit. Platinum electrodes situated in the wall of capillary S were used to stimulate the preparation at 1 min. intervals. Action potentials were picked up by spaced electrodes in compartment B and recorded by means of a cathode ray oscilloscope in the usual way.

The second cell (Fig. 3a and b) was designed to make possible the recording of X-ray diffraction patterns during swelling and extraction of the tissue by lipid solvents. It was essentially a small distillation flask, fitted with windows W made of thin polythene. The solvent was heated by a small electric heater H coiled round the bottom of the cell. The vapour condensed on a cold-finger type of condenser C, fitted into the top of the cell, the solvent running back down the nerve specimen hanging from the bottom of the condenser. Polythene windows were found suitable for use with alcohol, ether and acetone, but were attacked by such solvents as benzene and chloroform.

**Preparation of Frog Material.**—As in previous experiments, the animals were stunned, decerebrated, and the sciatic nerve freed as gently as possible throughout its course. In the preparation of some specimens, the whole of the spinal column with the sciatic nerve attached by its roots was removed, the nerve being cut between ligatures at a point just short of its entry into the gastrocnemius muscle. In experiments involving the recording of action potentials parallel with X-ray diffraction patterns, the gastrocnemius muscle was dissected out, together with the sciatic nerve. In this case the nerve was ligatured close to its spinal roots, and stimulated some distance away from this ligature.

**Procedure.**—The general practice was to examine diffraction patterns of fresh and fully dried nerve in detail, using filtered radiation, and then to follow the intermediate stages of drying as frequently as time of exposure would permit (usually at 10 to 20 min. intervals). An attempt was also made to distinguish between the various long-spacing diffraction bands of fully dried nerve by observing their varying behaviour upon treatment with a number of solvents. The effect of rewetting of partially or fully dried tissue by Ringer solution was also studied. The following notes give some details of procedure.

(a) **FRESH NERVE.**—Specimens were either mounted in the irrigation cell and kept moist by Ringer solution in the way already described, or sealed in a thin-walled glass capillary tube containing Ringer solution. An alternative was to place the specimen in a capillary tube dipped into a vessel containing Ringer solution. Slight tension was usually applied to the specimen by attaching a weight of 1 to 2 g.

(b) **FULLY DRIED NERVE.**—Specimens, dried to constant weight in air or *in vacuo* were examined either in the open camera or *in vacuo*.

(c) **INTERMEDIATE STAGES OF DRYING.**—Two different procedures were employed. One was to ligature a length of nerve at either end and mount it across a wire loop; serial exposures were then taken while the specimen was drying in the open camera. In other experiments the nerve was mounted in the irrigation cell. Irrigation was discontinued by closure of valve Y, and the rate of drying in chamber C controlled as far as possible by passing a stream of air of constant humidity through the compartment. In some of the experiments, action potentials were recorded at the same time. Time of exposure (at specimen to film distances of 20 cm.) varied between 10 and 20 min.

(d) **REWETTING OF PARTIALLY OR FULLY DRIED NERVE.**—Rewetting was effected immediately after recording the diffraction pattern, either by soaking the material in Ringer solution for 30 min., or alternatively,

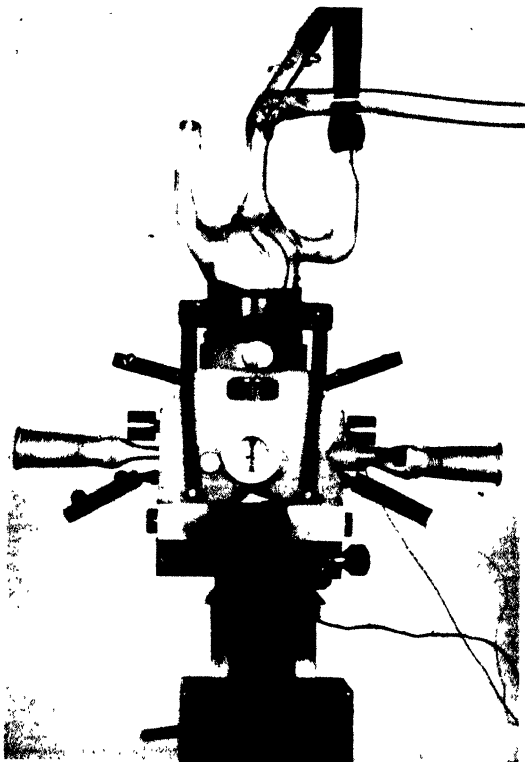


FIG. 2.-Irrigation cell. Front view.

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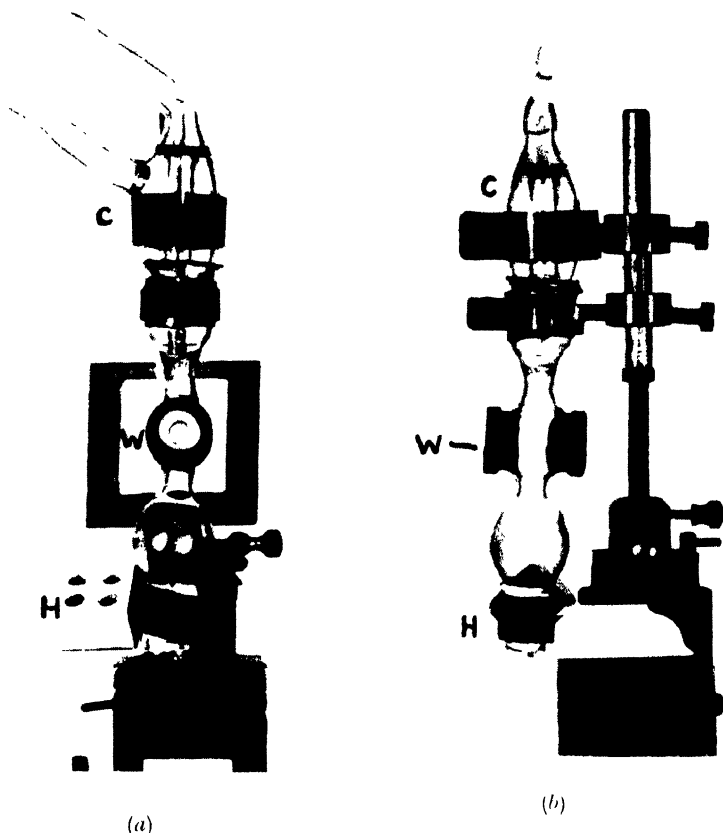


FIG. 3.—Cell used for observing the effects of lipid solvents upon nerve preparations.

(a) Front view.

(b) Side view.

C — Condenser. W — Polythene windows for passage of X-rays. H — Electric heater.

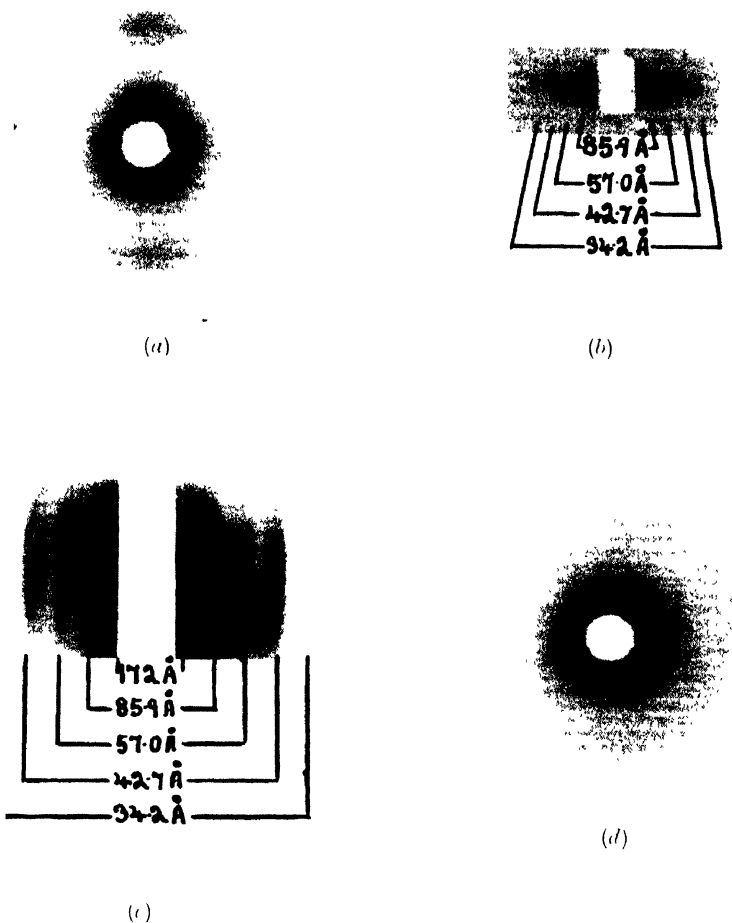


Fig. 4. X-ray diffraction patterns obtained from fresh nerve.

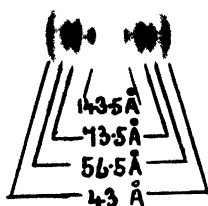
(a) With incident beam perpendicular to fibre direction. Preparation under tension exerted by weight of 1.5 g. Note equatorial spots at  $15.0 \pm 0.2 \text{ \AA}$  and ring at  $4.7 \pm 0.1 \text{ \AA}$  showing meridional intensification.

(b) With incident beam perpendicular to fibre direction. Preparation under tension exerted by weight of 1.5 g.

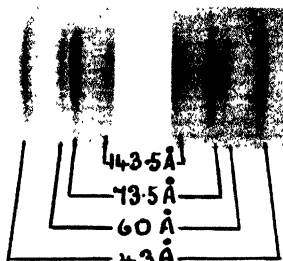
(c) With incident beam parallel to fibre direction. No tension.

(d) With incident beam perpendicular to fibre direction. No tension.

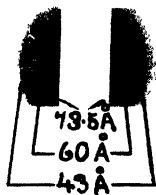




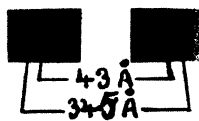
(a)



(b)



(c)



(d)

FIG. 5.—X-ray diffraction patterns obtained from fully dried nerve. (a), (b), and (d) with incident beam perpendicular to fibre direction; (c) with incident beam parallel to fibre direction.

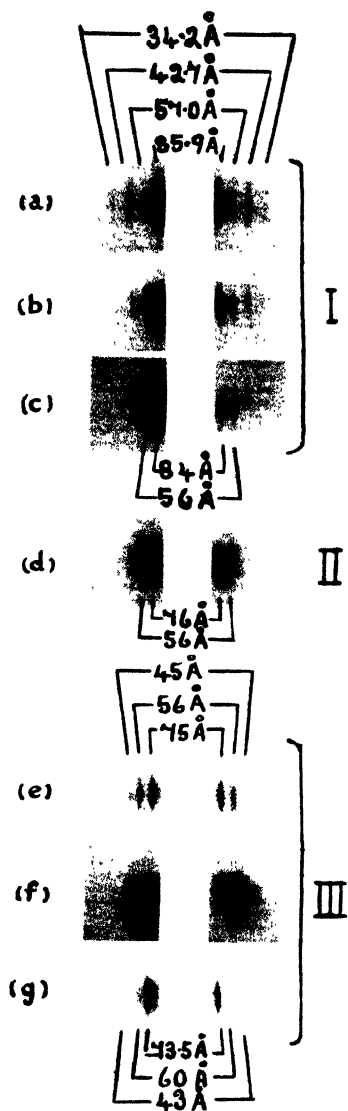


FIG. 7. -Examples of diffraction patterns obtained during intermediate stages of drying. Incident beam perpendicular to fibre direction.

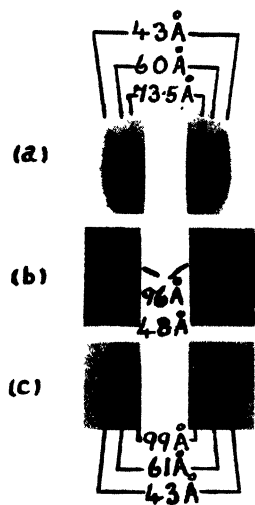


FIG. 8. X-ray diffraction patterns obtained on rewetting (and subsequent redrying) of a fully dried nerve preparation. Incident beam perpendicular to the fibre direction.

- (a) Fully dried nerve.
- (b) Fully dried nerve after rewetting with Ringer solution.
- (c) Pattern obtained upon redrying.

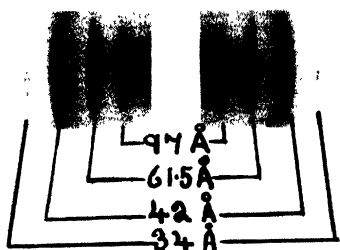


FIG. 9. X-ray diffraction pattern obtained from specimen dried to constant weight and subsequently left exposed to air for 3 months.

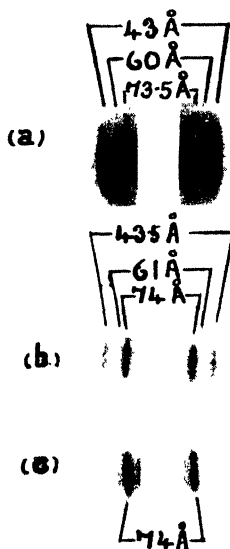


FIG. 10. Effect of ether on the X-ray diffraction pattern of fully dried nerve.

- (a) Fully dried nerve.
- (b) Fully dried nerve after being suspended over ether in the distillation cell for 12 hr.
- (c) Pattern obtained during the refluxing of ether over the specimen. The exposure (of 15 mm. duration) was started after 5 min. of preliminary refluxing.

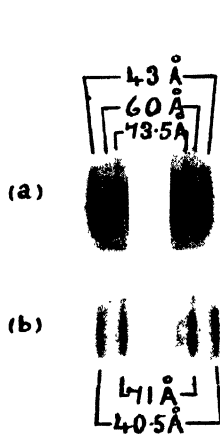


FIG. 11—Effect of acetone on the diffraction pattern of fully dried nerve.

- (a) Fully dried nerve.
- (b) Pattern obtained during the refluxing of acetone over the specimen. The exposure (of 15 min. duration) was started after 5 min. of preliminary refluxing.

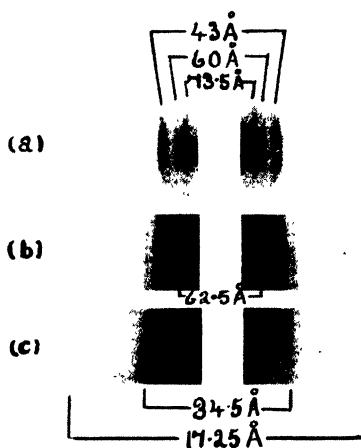


FIG. 12—Effect of alcohol on the diffraction pattern of fully dried nerve.

- (a) Fully dried nerve.
- (b) Pattern obtained from a dried nerve specimen which had been treated with alcohol in the distillation cell for 5 min. and subsequently dried for 12 hr.
- (c) Procedure as in (b), followed by gentle treatment with alcohol in distillation cell. Duration of exposure 15 min.



by starting the flow of Ringer solution along the nerve in compartment C. The changes in diffraction pattern were followed in stages.

(e) TREATMENT OF DRIED NERVE BY LIPID SOLVENTS AND THEIR VAPOURS.—Some of these experiments were carried out in the irrigation cell by first drying the nerve in compartment C, and then adding solvent vapours to the air stream passing through the compartment. Other experiments were carried out by refluxing the specimen in the distillation cell, several exposures being made whilst distillation was in progress.

## Results

In the Figures which follow, any dimension given for a particular diffraction spacing represents the mean value derived from a number of experiment, carried out under slightly varying conditions. The maximum variation observed in these experiments is stated.

(a) FRESH NERVE IN RINGER SOLUTION.—Diffraction patterns were obtained from 25 preparations. Of these, five were examined at a specimen to film distance of 4.5 cm. and the remainder at 20 to 40 cm. With the incident X-ray beam perpendicular to the fibre direction, the diffraction pattern showed a broad diffuse ring, with marked meridional intensification at  $4.7 \pm 0.1 \text{ \AA}$  and equatorial spots at  $15.0 \pm 0.2 \text{ \AA}$  (Fig. 4a) and further equatorial spots at  $34.2 \pm 0.5 \text{ \AA}$ ,  $42.7 \pm 1.0 \text{ \AA}$ ,  $57.0 \pm 1.5 \text{ \AA}$  and  $85.9 \pm 1.5 \text{ \AA}$  (Fig. 4b). An additional faint band at approximately  $172.0 \text{ \AA}$  was also detected (Fig. 4c). With the incident beam parallel to the fibre direction (Fig. 4c) the diffraction bands furnished the same dimensions but did not show the same degree of orientation. The application of tension increased the orientation of the  $4.7 \pm 0.1 \text{ \AA}$  and  $15.0 \pm 0.2 \text{ \AA}$  spacings (compare Fig. 4a and 4d), but left the longer spacings relatively unaffected.

(b) FULLY DRIED NERVE.—Twenty specimens were examined, five a specimen to film distances of 4.5 cm. and the remainder at 20 to 40 cm. The patterns did not differ materially from each other, provided drying was complete. The short spacing ring at  $4.7 \pm 0.1 \text{ \AA}$  was broad, diffuse and showed no localized intensification. In two of the patterns, this spacing showed signs of splitting into several rings. All long spacing patterns showed intense bands at  $73.5 \pm 1.5 \text{ \AA}$  and  $43.0 \pm 1.0 \text{ \AA}$  (Fig. 5a and b). With the beam perpendicular to the fibre direction (Fig. 5a and b) the former spacing showed equatorial accentuation comparable with that seen in fresh nerve; the latter showed only very slight orientation. No orientation could be detected in patterns obtained by irradiation parallel to the fibre direction (Fig. 5c). In 13 of the patterns there was a faint band at  $60.0 \pm 1.0 \text{ \AA}$  (Fig. 5b). The remaining 11 showed a more intense band at  $56.5 \pm 1.0 \text{ \AA}$  (Fig. 5a). The orientation of these bands was very similar to that of the  $73.5 \pm 1.5 \text{ \AA}$  band, but by carefully heating the specimen to about  $90^\circ$  it was possible to destroy the orientation of the  $56.5$  or  $60 \text{ \AA}$  bands without affecting the one at  $73.5 \pm 1.5 \text{ \AA}$ . In six of the more intense patterns produced by exposures between 40 and 60 min., a faint band was observed at  $34.5 \pm 1.0 \text{ \AA}$  (Fig. 5d). In six others a band of similar intensity was seen at  $37.5 \pm 1.0 \text{ \AA}$ , whilst in one case both bands were seen in the same pattern. As far as could be judged, these bands showed little preferred orientation. In three long spacing patterns an additional faint band at  $143.5 \pm 3.0 \text{ \AA}$  could be resolved (see Fig. 5a and b).

(c) INTERMEDIATE STAGES OF DRYING.—Ten series were examined, involving 79 exposures. The beam was kept perpendicular to the fibre direction throughout. Fig. 6 summarizes the findings in a typical experiment, and some typical diffraction patterns are shown in Fig. 7. A similar sequence of events was observed in all experiments, though the rate necessarily varied from one series to another. By following the changes

in position and intensity of the individual bands, three stages could be conveniently distinguished. These are summarized in Table I.

(d) TREATMENT OF FULLY OR PARTLY DRIED NERVE WITH RINGER SOLUTION.—Specimens treated with Ringer solution at any point during stages I and II showed a return to what appeared to be their original pattern. The usual sequence of changes could be detected upon re-drying the material. No such reversibility was observed with preparations dried to stage III. Instead, the pattern obtained upon rewetting with Ringer solution showed two well-defined and intense bands at  $48.0 \pm 1.0 \text{ \AA}$  and  $96.0 \pm 3.0 \text{ \AA}$  (Fig. 8b). On re-drying the material, an unusual dried nerve pattern, showing three bands of similar intensities at  $43.0 \pm 1.0 \text{ \AA}$ ,  $61.0 \pm 1.0 \text{ \AA}$  and  $99.0 \pm 3.0 \text{ \AA}$  could be seen (Fig. 8c). Each step in these experiments has been repeated six times.

It was also of interest that in the five experiments in which the action potential was recorded during a drying series, no response could be picked up in compartment B (Fig. 1) if drying had been allowed to

SUMMARY OF CHANGES IN DIFFRACTION PATTERN DURING DRYING

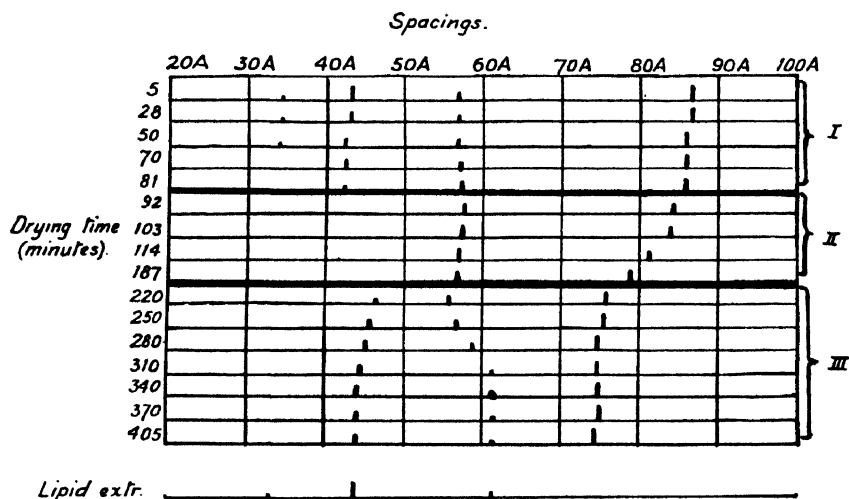


FIG. 6

proceed beyond stage II; the significance of this must at present remain an open question.

(e) THE TREATMENT OF FULLY DRIED NERVE WITH LIPID SOLVENTS AND THEIR VAPOURS.—Full details of these experiments will be published elsewhere, but a few photographs are reproduced to illustrate the varying behaviour of some of the diffraction bands on treatment with lipid solvents. Fig. 10 shows the effect of ether on the dried nerve patterns. Extraction in this experiment was slight, and the spacings characteristic of dried nerve could be made to re-appear on leaving the specimen exposed to air for a few hours. A slight loss in the orientation of the  $60.0 \pm 1.0 \text{ \AA}$  spacing, seen in the re-dried specimen, appeared to be the only detectable effect of this initial exposure to ether.

Similar reversibility was observed following treatment with acetone (Fig. 11). Treatment with alcohol, on the other hand, irreversibly removed the  $73.5 \pm 1.5 \text{ \AA}$  spacing (change (a) to (b) Fig. 12), though reversibility in a later phase ((b) to (c)) was maintained. Prolonged treatment with any of the solvents invariably resulted in the extraction of lipid material. The diffraction spacings of this material were of interest when compared with those of the original, and the residual, tissue.<sup>3, 4</sup>

TABLE I.—SUMMARY OF CHANGES IN DIFFRACTION SPACINGS OBSERVED DURING INTERMEDIATE STAGES OF DRYING

Diffraction Spacings Å: Fresh Nerve	Stage I	Stage II	Stage III	Diffraction Spacings Å: Fully Dried Nerve
4.7 ± 0.1	Loss of meridional intensification			4.7 ± 0.1
15.0 ± 0.2	Disappears	Not seen	Not seen	
34.2 ± 0.5	Disappears	Not seen	Faint bands at 34.5 ± 1.0 Å and/or at 37.5 ± 1.0 Å detected on long exposure	34.5 ± 1.0 and/or 37.5 ± 1.0
42.7 ± 1.0	Disappears	Not seen	Intense band, showing only slight equatorial orientation appears at 43.0 ± 1.0 Å	43.0 ± 1.0
57.0 ± 1.5	Position unchanged, but marked increase in intensity until it equals that of the 85.9 ± 1.5 Å band	Slight loss of orientation	Drastic decrease in intensity, ultimately replaced by a faint band at 60.0 ± 1.0 Å*	56.5 ± 1.0 or 60.0 ± 1.0
85.9 ± 1.5	Little change	Rapid shrinking of dimension; some fading of the band during the middle of the stage; ultimately replaced by intense band at 73.5 ± 1.5 Å	No further change	73.5 ± 1.5
172.0 ± 3	Changes could not be followed owing to long exposures required to record this spacing			143.5 ± 3.0

\* In 11 of the 24 fully dried nerve specimens examined, a moderately intense band at 56.5 ± 1.0 Å was seen instead of the faint band at 60.0 ± 1.0 Å.

### Discussion

The marked equatorial orientation of the long spacings in the diffraction patterns of fresh nerve, obtained with the beam perpendicular to the fibre axis, and the loss of such orientation in photographs taken parallel to it, suggests a radial orientation of the diffracting units with respect to this axis. Comparison of the position, and the relative intensities of the diffraction bands suggests that they may be related, and may in fact be orders of a fundamental spacing. Calculation from the dimensions given in Fig. 4b, would put this at 171.15 Å ( $d = \pm 0.41$  Å or a simple multiple of this figure). The 15.0 ± 0.2 Å spacing resembles the larger spacings so closely, both as regards definition and orientation that it may reflect a lower (11th or 12th) order of the same fundamental spacing. In this case, a unit cell, measuring approximately



$171 \times 4.7 \times 4.7 \text{ \AA}^3$  is suggested. Alternatively, the  $15.0 \pm 0.2 \text{ \AA}$  spacing may represent another dimension, perpendicular to the fibre direction. This would make for a unit cell of about  $171.0 \times 4.7 \times 15.0 \text{ \AA}^3$ . The effect of application of tension on the orientation of some of the diffraction bands of the fresh nerve pattern may reflect an improvement in the alignment either of the fibres within the nerve bundle or of some components within the structure of the myelin.

The pattern of fully dried nerve lends itself to a number of interpretations, of which only two will be mentioned. On the one hand it is possible to regard the diffraction spacings as representing once again orders of a fundamental spacing: with the exception of the  $56.5 \pm 1.0 \text{ \AA}$  bands, the dimensions observed ( $143.5 \pm 3.0 \text{ \AA}$ ,  $73.5 \pm 1.5 \text{ \AA}$ ,  $60.0 \pm 1.0 \text{ \AA}$ ,  $43.0 \pm 1.0 \text{ \AA}$ ,  $37.5 \pm 1.0 \text{ \AA}$  and  $34.5 \pm 1.0 \text{ \AA}$ ) correspond fairly well to the 2nd, 4th, 5th, 7th, 8th, and 9th orders of a long spacing of about  $300.0 \text{ \AA}$ —a dimension which might conceivably arise from a unit measuring  $342 \text{ \AA}$  ( $2 \times 171 \text{ \AA}$ ) by the loss of water. Certain facts, however, are not in keeping with this interpretation. The  $57.0 \pm 1.5 \text{ \AA}$  and  $85.9 \pm 1.5 \text{ \AA}$  bands, seen at the beginning of stage II, would, on the above explanation, correspond to 6th and 4th order diffractions of a  $342 \text{ \AA}$  unit; yet their behaviour throughout this stage appeared independent, the  $85.9 \text{ \AA}$  band shrinking rapidly, to be ultimately replaced by a spacing at  $73.5 \pm 1.5 \text{ \AA}$ , whereas  $57.0 \pm 1.5 \text{ \AA}$  band remained unchanged. The intermediate changes observed during stage II left little doubt about the gradual shift of the  $85.9 \pm 1.5 \text{ \AA}$  band, and its relation to the one seen at  $73.5 \pm 1.5 \text{ \AA}$ . A similar independence between spacings was observed during stage III where (in 13 instances) the  $57.0 \pm 1.5 \text{ \AA}$  band shifted to  $60.0 \pm 1.0 \text{ \AA}$ , no change being seen in the spacings at  $43.0 \pm 1.0 \text{ \AA}$  and  $73.5 \pm 1.5 \text{ \AA}$ . The difference in orientation between the  $43.0 \pm 1.0 \text{ \AA}$  and  $73.5 \pm 1.5 \text{ \AA}$  bands (Fig. 5a) is contrary to what one would expect with orders of the same fundamental spacing and suggests that these, too, may be independent. This is borne out by the effect of lipid solvents. The lipids extracted from nerve, when dried, give spacings which correspond very closely to the  $34.5 \pm 1.0 \text{ \AA}$ ,  $43.0 \pm 1.0 \text{ \AA}$  and  $60.0 \pm 1.0 \text{ \AA}$  spacings of the dried nerve pattern.<sup>4, 5</sup> Furthermore, in partial extraction of dried nerve by solvents, it is usually possible to detect a decrease in intensity in one or more of these bands parallel with the appearance of corresponding bands in the lipid extracts.<sup>3, 5</sup> A possible explanation of the three lower spacings ( $34.5 \pm 1.0 \text{ \AA}$ ,  $43.0 \pm 1.0 \text{ \AA}$  and  $60 \pm 1.0 \text{ \AA}$ ) would therefore be that they correspond to first-order diffractions from groups of lipids which have broken away from the original structure on drying. The loss of orientation in the case of the  $43.0 \pm 1.0 \text{ \AA}$  and  $34.5 \pm 1.0 \text{ \AA}$  spacings is in keeping with this explanation. No such loss of orientation could be detected in the  $56.5 \pm 1.0 \text{ \AA}$  (or  $60 \pm 1.0 \text{ \AA}$ ) spacings, though their behaviour on heating differentiated them from the well-orientated spots seen at  $73.5 \pm 1.5 \text{ \AA}$ . This latter spacing ( $73.5 \pm 1.5 \text{ \AA}$ ) is higher than any yet obtained from a single lipid (or mixture of lipids) in the dry state.<sup>3-6</sup> Yet it is of interest that any change leading to a modification of this spacing (as, for example, treatment with lipid solvents or Ringer solution, and subsequent re-drying) was invariably accompanied by

<sup>4</sup> Bear, Palmer and Schmitt, *J. Cell. Comp. Physiol.*, 1941, 17, 355.

<sup>5</sup> Elkes and Finean (unpublished data).

<sup>6</sup> Schmitt and Palmer, *Cold Spring Harb. Symp. Quant. Biol.*, 1940, 8, 94.

an increase in the intensity of one or more of the lower spacings; and that these spacings in turn were susceptible to (i.e. could be weakened or removed by) treatment with lipid solvents. Modification of the unit responsible for the  $73.5 \pm 1.5$  Å spacing would therefore appear to result in a further separation of lipid material. This suggests that the original unit may be built of lipid and non-lipid components. The latter may be protein.<sup>1</sup>

Such a lipid-lipo-protein system may therefore serve as a possible basis for a second interpretation of the pattern of fully dried nerve, the  $73.5 \pm 1.5$  Å,  $143.5 \pm 3.0$  Å and  $37.5 \pm 1.0$  Å spacings being related as different orders of diffraction to a residual lipo-protein unit, whereas the  $34.5 \pm 1.0$  Å,  $43.0 \pm 1.0$  Å and  $56.5 \pm 1.0$  Å or  $60 \pm 1.0$  Å spacings could be due to the existence of separate phases of lipid. The persistent equatorial orientation of the  $73.5 \pm 1.5$  Å spacing may conceivably be due to an orienting protein framework, with which the lipid retains van der Waals' association in the dry state. To a lesser extent this may also apply to the equatorial orientation of the  $56.5 \pm 1.0$  Å or  $60 \pm 1.0$  Å spacings.

The intermediate changes observed during drying would appear to lend support to the above view. The rapid fading of the  $42.7 \pm 1.0$  Å and  $34.2 \pm 0.5$  Å bands early during stage I and the striking increase in the intensity of the  $57.0 \pm 1.5$  Å band may reflect a re-arrangement of the lipo-protein structure, or alternatively, a breaking-up of the original lipo-protein into two components characterized by bands at  $57.0 \pm 1.5$  Å and  $85.9 \pm 1.5$  Å respectively. The independent behaviour of the two bands during stage II favours the latter explanation. The rapid shrinking of the  $85.9 \pm 1.5$  Å spacing during this stage and its ultimate replacement by a band at  $73.5 \pm 1.5$  Å may indicate the removal of water layers either from within or from between the lipo-protein layers, whereas the slight disorientation observed in the  $57.0 \pm 1.5$  Å band may be in keeping with a possible separation of lipid during this phase. The reversibility of the changes during stages I and II may reflect the existence of preformed units measuring approximately 85.9 Å and 57.0 Å. The changes noted in stage III, on the other hand, would seem to indicate an irreversible breakdown. The appearance of a strong, poorly orientated spacing at  $43.0 \pm 1.0$  Å may reflect this change.

A more detailed interpretation of structure may be possible when more information regarding the identities and properties of the individual lipids and proteins is available. Such information is being sought by chemical analysis, spectrography, and by observing the effect of various enzymes upon this lipo-protein.

We wish to thank Prof. E. K. Rideal, F.R.S., for the facilities generously provided to us at the Davy Faraday Laboratory of the Royal Institution, London; Prof. A. C. Frazer for his interest in this work; Dr. R. W. H. Small for much helpful discussion and criticism; Mr. H. Smith for valuable technical assistance; and the Medical Research Council and the Sir Halley Stewart Trust for financial support.

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## GENERAL DISCUSSION\*

**Prof. Frey-Wyssling (Zürich)** said: The chloroplasts shown in the electron micrograms have been broken down by distilled water, as they must be washed to remove the salts which would contaminate the electron micro pictures by crystallizing. Of course, it should be possible to separate the granules of the chloroplast and the macromolecules, but seemingly the high lipid content interferes unfavourable in crude chloroplast preparations. Grana suspension out of chloroplasts have not been obtained as yet. What has been described as such has been seriously criticised.

**Prof. W. T. Astbury (Leeds)** said: I should like to ask where collagen comes into this story. Are there no indications of the presence of collagen in the myelin sheath even after extracting the lipid or lipo-protein which might conceivably overwhelm the collagen reflections in the fresh nerve?

**Dr. J. B. Finean (Birmingham)** said: X-ray diffraction patterns of nerve preparations, particularly of dried or extracted material, usually show a band at about 2.8 Å, but the intensity of this band is very low when compared with the 4.7 Å ring. The 2.8 Å spacings may be due to collagen, but that the 4.7 Å ring is mainly due to lipids is indicated by the fact that its intensity is much reduced when lipid is extracted from the preparation. It is, however, difficult to consider the 2.8 Å spacings in terms of a possible protein component of myelin, since apart from the proteins of axoplasm, the material also contained the proteins of the peri- and endo-neurium. The latter almost certainly contains collagen, and variations in its content have been observed during various phases of Wallerian degeneration.<sup>1</sup> The dominant diffraction pattern of nerve is certainly due to ordered lipid material.

**Dr. R. W. H. Small (Birmingham)** said: Some points arise from Dr. Finean's paper: firstly, the sharpness of the bands in the dried nerve photograph attributed to pure lipid indicate that there must be complete detachment of the lipid from the protein framework. What remains then? There are two possibilities: that the 142 and 73 Å bands are due to a lipo-protein system of a more strongly bound type than that which gave rise to the free lipid, or, alternatively, that the residual bands can be interpreted in terms of a pure protein system. The 4.7 Å meridionally accentuated band is strongly suggestive of the protein backbone spacing.

**Dr. J. B. Finean (Birmingham)** said: Points in favour of the explanation that the 143 Å and 73.5 Å bands reflect a lipid or lipo-protein component and not a protein alone are, firstly, the complete disappearance of the 73.5 Å and 143.0 Å bands on refluxing the preparation with alcohol for 5 min., and secondly, the fact that the disappearance of this band is accompanied by an increase in the intensity of the lower spacings at 34.5 Å, 43.0 Å and 56.5 Å, and 60.0 Å. These latter spacings are of the order seen in lipid extracts of nerve and the increase in intensity may suggest a setting free of lipid from the component giving rise to the 143 Å and 73.5 Å bands. The 4.7 Å band could be due to either a lipid or a lipo-protein, but the fact that lipids alone give rise to a 4.7 Å ring, and that this ring is greatly reduced in intensity by treatment of the nerve material with lipid solvents, indicates that lipids probably make the larger contribution.

**Dr. K. G. A. Pankhurst (London) (communicated)**: I should like to ask Dr. Elkes and Dr. Finean whether they have attempted to vary temperature or humidity during the drying of their preparations. A few years ago, when studying the effect of heat and humidity on collagen and gelatin, I found that considerable re-orientation ("incipient shrink-

\* On two preceding papers.

<sup>1</sup> Abercrombie and Johnson, *J. Neurol. Neurosurg. Psych.*, 1946, 9, 113.

age") of the protein took place if the specimen was kept at high humidities during drying.<sup>2</sup>

**Dr. J. B. Finean** (*Birmingham*) said: The effects of temperature on these drying changes have not as yet been studied in detail. However, in two preliminary experiments, in which the preparations were dried at 50° and 60° C respectively, the diffraction patterns observed differed considerably from those seen with nerve dried at room temperature. The main differences were seen in spacings higher than 70 Å, and similar differences were observed in fresh nerve preparations heated to 50° or 60° C in Ringer solution and dried subsequently. Such modification in diffraction patterns could be due to changes in the protein component brought about by heating, though further detailed work will be needed to elucidate this question. Special thermostatically controlled irrigation and drying chambers have been constructed, and will be used for this purpose.

<sup>2</sup> *Nature*, 1947, **159**, 538.

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## THE PROTOPLASMIC MEMBRANE REGARDED AS A LIPO-PROTEIN COMPLEX

By H. L. BOOIJ

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The complex theory of permeability of Bungenberg de Jong tries to reconcile the often conflicting older theories. The protoplasmic membrane is considered to be a tri-complex between protein, phosphatide and a cation. Experiments on yeast show that the cation is often exchangeable, which results in an altered permeability and consequently in a changed rate of fermentation. Organic non-electrolytes may concentrate in the membrane and thus influence permeability. It is supposed that the apolar part of the membrane forms a continuous layer which will, however, be a "statistical sieve" as in this mono-, di-, or pauci-molecular film one may not neglect the movements of the molecules themselves. The variation of the membrane constituents may account for the fact that different organisms, organs or cells show very divergent rates of permeability for various substances.

Before considering the structure of the protoplasmic membrane, it is necessary to review the facts which the study of permeability of living organisms has revealed. Many authors have tried to combine these facts into a theory on permeability, which has resulted in a multitude of theories, each describing part of the phenomena. The two main principles deduced from experiments on permeability are:

(a) The permeation of a substance is governed by its selective solubility in membrane compounds (Overton<sup>1</sup> finding a correlation between the rate of permeation and the solubility in oil, suggested that these membrane compounds are lipids).

(b) The membrane acts like a sieve or filter with pores of different widths, such that large molecules cannot enter the protoplasm (or only with great difficulty); from this point of view the molecular volume is the main principle in permeation.<sup>2, 3</sup>

<sup>1</sup> Overton, *Vjschr. naturf. Ges. Zürich*, 1895, **40**, 159.

<sup>2</sup> Ruhland and Hoffmann, *Planta*, 1925, **1**, 1.

<sup>3</sup> Traube, *Arch. Anat.*, 1867, 87.

Now it was found<sup>4, 5, 6, 7</sup> that no organism can be regarded as an example of only one of these two principles, the situation being such that the organisms may be placed in a series with permeability phenomena ranging from very much of principle (a) and much less of principle (b) to exactly the reverse. Thus every conception of the structure of the protoplasmic membrane must involve not only a combination of the principles mentioned, but also the possibility of variation. Then we arrive at theories like the mosaic theory (Nathanson<sup>8</sup>), and the lipid-sieve theory (Collander<sup>9</sup>). Clowes,<sup>10</sup> in framing his emulsion theory, drew the attention to yet another phenomenon shown by the membrane, viz. the influence of ions and ion-antagonism. Practically everyone agrees that at least some lipids are to be found in the membrane, be it only in patches (Nathanson,<sup>8</sup> Wolpers<sup>11</sup>), in a unimolecular (Winkler<sup>12, 13</sup>), a bimolecular (Bungenberg de Jong<sup>14, 15</sup>) or a paucimolecular layer (Danielli and Davson<sup>5</sup>). This state of affairs justifies a closer study of the physical chemistry of lipids.

**Complex relations of phosphatides.**—The influence of ions on the membrane makes it likely that among the lipoids only the strongly polar ones are the main building material. Less polar material will then be incorporated in the membrane; this may result in an extra strengthening effect and in a lowering of permeability for water. Therefore it is obvious that we must study first the "backbone" of the membrane, and this means that phosphatides must be investigated. Of the many methods used, I will select only the study of the complex (electrical) relations which was inaugurated by Bungenberg de Jong.\* We must look at the main conclusions deduced from a simpler system where complex relations play a great part, e.g. the gum arabic-gelatin coacervate.<sup>16</sup> A word of caution must be said here. The fact that we study complex relations on a coacervate does not mean that such relations are present in coacervates only, and it certainly does not mean that we consider the protoplasmic membrane to be a coacervate.

At a certain pH (< 4.8) gelatin will be positively charged, while gum arabic will be negative at much lower values of pH, becoming uncharged at pH = 2.7. So at a certain interval of pH the two colloids will be oppositely charged and after mixing the two a separation into two layers is observed. The colloid-rich layer is most concentrated when the mutual attraction is at a maximum (Fig. 1).

<sup>4</sup> Brooks and Moldenhauer Brooks, *The Permeability of Living Cells* (Borntraeger, Berlin, 1941).

<sup>5</sup> Davson and Danielli, *Permeability of Natural Membranes* (Cambridge, University Press, 1943).

<sup>6</sup> Gellhorn and Régner, *La perméabilité en physiologie et en pathologie générale* (Masson, Paris, 1936).

<sup>7</sup> Höber, *Physical Chemistry of Cells and Tissues* (Blakiston, Philadelphia, 1945).

<sup>8</sup> Nathansohn, *Jahrb. wiss. Bot.*, 1904, **39**, 607.

<sup>9</sup> Collander and Bärklund, *Acta Botan. Fennica*, 1933, **11**, 1.

<sup>10</sup> Clowes, *J. Physic. Chem.*, 1920, **20**, 407.

<sup>11</sup> Wolpers, *Naturwiss.*, 1941, **29**, 416.

<sup>12</sup> Winkler, *Thesis* (Leiden, 1938).

<sup>13</sup> Winkler and Bungenberg de Jong, *Archiv. Néerl. physiol.*, 1940, **25**, 431, 467.

<sup>14</sup> Bungenberg de Jong and Bonner, *Protoplasma*, 1935, **24**, 198.

<sup>15</sup> Bungenberg de Jong and Saubert, *ibid.*, 1937, **28**, 352.

\* Practically all of Bungenberg de Jong's investigations will be found in Kruyt, *Colloid Science II* (Elsevier, Amsterdam, 1949).

<sup>16</sup> Bungenberg de Jong and Dekker, *Kolloidchem. Beih.*, 1935, **43**, 143, 213.

The most characteristic property of such a coacervate is the fact that it will dissolve under the influence of appropriate concentrations of neutral salts, which salts arrange themselves according to the so-called "double valency rule" (Fig. 2).

A substance in which positive and negative charges are combined must in principle have the ability to coacervate (or flocculate if the

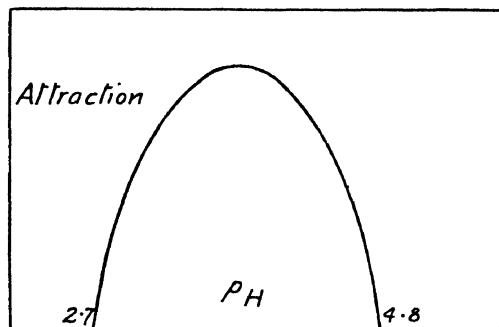


FIG. 1.—Mutual attraction of gelatin and gum arabic at different values of pH.

"coacervate" has a very low content of water). This flocculation or coacervation will be strongest, of course, when the number of positive charges equals that of the negative ones, while neutral salts will cause the flocculation to disappear according to the double valency rule. This was shown to be the case with the flocculation of serum globulin at its isoelectric point.<sup>17</sup>

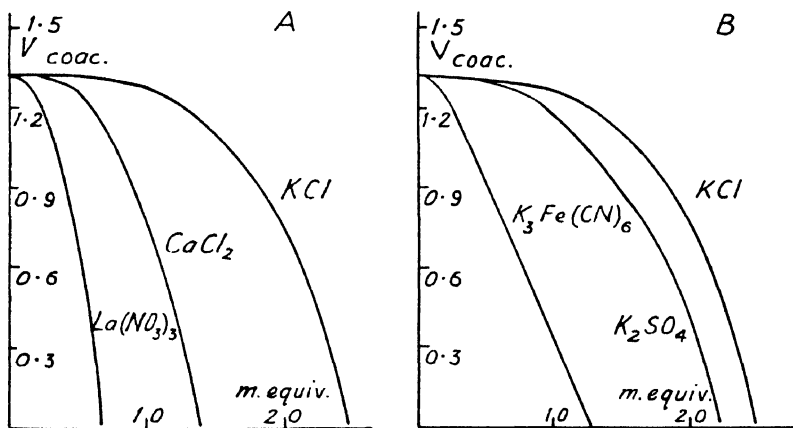


FIG. 2.—Double valency rule. Disappearance of a gelatin-gum arabic coacervate under the influence of neutral salts (A = valency rule for the cations; B = valency rule for the anions).

Lecithin too belongs to this class of substances and the same phenomena would be expected here. Now naturally occurring lecithin shows a negative charge, suggesting that part of the choline groups are split off. It is possible to reverse the charge of lecithin by adding an adequate amount of salt. Then it follows logically that coacervation (flocculation) is at its maximum at zero charge (Fig. 3).

<sup>17</sup> Bungenberg de Jong, Dekker and Winkler, *Rec. trav. chim.*, 1934, **53**, 607.

As the reversal of charge is a consequence of the attraction between the cation added and the negative group of the colloid it must be expected that the various cations will show a different behaviour. This leads to the investigation of the concentrations of various cations needed to reverse the charge of the acidic colloids (the so-called "ionic spectra"). Teunissen<sup>18, 19, 20</sup> found that the various colloids can be divided into three groups which show different ionic spectra. The decisive factor is the nature of the negative group in the colloids; so we can distinguish carboxyl, sulphate and phosphate colloids, each of which is characterized by a specific sequence of the cations. To mention but one example; in phosphate colloids he found the sequence Li,

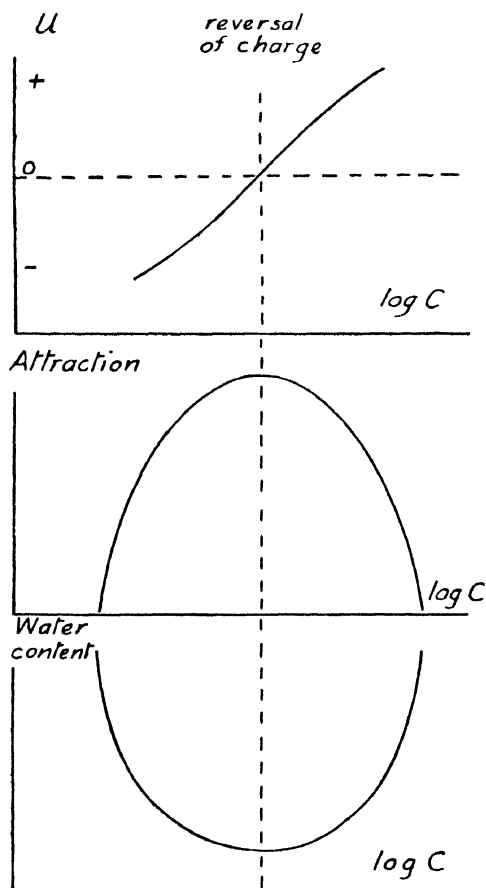


FIG. 3.—The attraction of the molecules of lecithin is at a maximum (i.e. the water content shows a minimum) at the zero-point of charge.

Na, K; in carboxyl and sulphate colloids the order is just the reverse, i.e. K, Na, Li. When a special sequence of cations is found in a certain biological phenomenon it might be concluded that a certain colloid plays a great part.

<sup>18</sup> Bungenberg de Jong and Teunissen, *Kolloidchem. Beih.*, 1938, 47, 254.

<sup>19</sup> Teunissen, *Thesis* (Leiden, 1936).

<sup>20</sup> Teunissen and Bungenberg de Jong, *Kolloidchem. Beih.*, 1938, 48, 33.

When we now try to give a schematic picture of a lecithin "coacervate" at the point of zero charge we obtain Fig. 4. Evidently such a lecithin structure in reality comprises three components and might be called a tricomplex system (in contrast to the gum arabic-gelatin coacervate, which is a bicomplex). We further ask whether some of

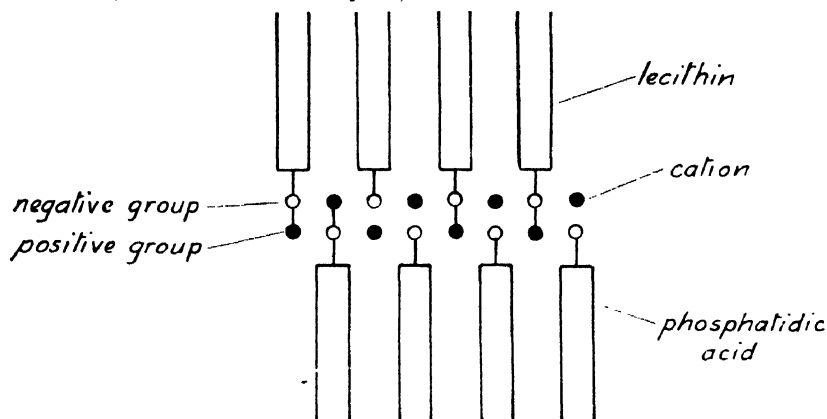


FIG. 4.—In a "coacervate" (flocculate) or natural lecithin three components will be found.

the components of this tricomplex system might not be changed for other substances. This question may be answered in the affirmative. A good example of tricomplex systems was given by Bungenberg de Jong and Rering<sup>21</sup> who added neutral salts to a mixture of lecithin and carrageen (a sulphate colloid). It then appeared that a tricomplex

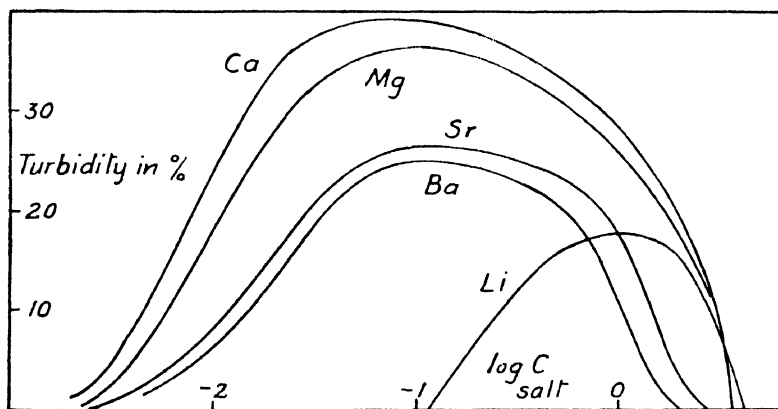


FIG. 5.—The formation of a tricomplex flocculation in a mixture of lecithin and carrageen under the influence of the alkaline earth cations.

is formed in salt concentrations which are not able to flocculate each of the colloids separately (Fig. 5). Tricomplex formation is strongest when the concentrations of the cations needed for reversal of charge of each of the colloids lie far apart (Fig. 6). Generally speaking, tricomplex formation is possible when we have an amphoteric ion in combination with an anion and a cation (one of which may be a colloid),

<sup>21</sup> Bungenberg de Jong and Rering, *Proc. K. Akad. Wetensch.*, 1942, **45**, 697, 705, 713.



with the restriction that the concentration must be such that bicomplex formation does not occur.

This idea leads us to the assumption that the protoplasmic membrane is built up from phosphatides and protein (which is an old view, of course) and that these substances are combined with a cation (presumably  $\text{Ca}^{++}$ ) to a tricomplex system. Let us now examine whether biological phenomena furnish some arguments in favour of this hypothesis.

**Electrical Relations in the Protoplasmic Membrane.**—The point of view developed above suggests that the cation in the membrane is exchangeable with another cation, which may change the electrical attraction within the polar part of the membrane, and thus may alter the cell permeability. This might be the basis of the different behaviour of  $\text{Ca}^{++}$  and  $\text{K}^+$  in many biological phenomena. De Haan<sup>22</sup> measured the influence of  $\text{NaNO}_3$ ,  $\text{Ca}(\text{NO}_3)_2$  and  $\text{Co}(\text{NH}_3)_6\text{Cl}_3$  on the permeability of *Allium cepa* cells. The experiment with  $\text{NaNO}_3$

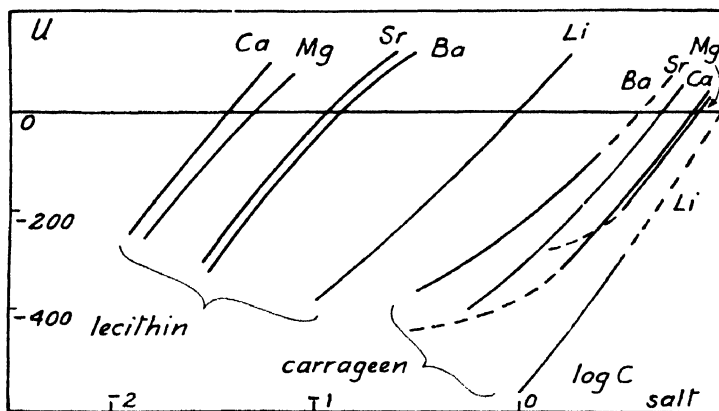


FIG. 6.—Ionic spectra (alkaline earth cations) of lecithin and carrageen. Tricomplex formation is strongest when the concentrations of zero charge lie far apart.

showed only an increase of permeability at high concentrations, whereas with  $\text{Ca}(\text{NO}_3)_2$  and  $\text{Co}(\text{NH}_3)_6\text{Cl}_3$ , permeability first decreases and afterwards increases again. The minimum of permeability lies at a lower concentration for the 3-valent cation than for the 2-valent one. One would expect that this minimum represents the zero-point charge of the membrane. The phenomenon of hypertonic haemolysis (haemolysis in high salt concentrations) is clearly related to the experiment mentioned; in high concentrations of neutral salt the tricomplex relations will be weakened considerably (according to the double valency rule). Winkler<sup>12, 13</sup> showed that pig's erythrocytes and stomata have a maximal stability at the isoelectric point and that this stability decreases according to the double valency rule when neutral salts are added. So positive and negative colloids must be present within the membrane. From the ionic spectrum of the erythrocytes he concluded that a phosphate colloid plays a dominant part. This must be lecithin, while the other component is the protein stromatin. Cations complete the tricomplex structure of the membrane. \*

The ready exchangeability of the cations in the protoplasmic mem-

<sup>22</sup> De Haan, *Protoplasma*, 1935, **24**, 186.

brane was shown by experiments in our laboratory.<sup>1</sup> The influence of a multitude of salts on the fermentation of sugar by bakers' yeast was investigated and found to be greatly influenced by the cations added (see e.g. Fig. 7).

Some cations stimulate fermentation, others decrease it to a certain percentage. (If the concentration is higher than  $\pm 0.5$  N, osmotic factors play a great part.) This leads to the idea that the normally occurring cation in the membrane is replaced by another one. Then according to the nature of the cation added two things may occur :

- (i) if the complex relations are strengthened permeability for sugar will decrease ;
- (ii) in the reverse case, a stimulating action of the cation added will be observed.

If all normal cations of the membrane are replaced (these normal cations presumably being  $\text{Ca}^{++}$ , as  $\text{Ca}^{++}$  has no influence on fermentation)

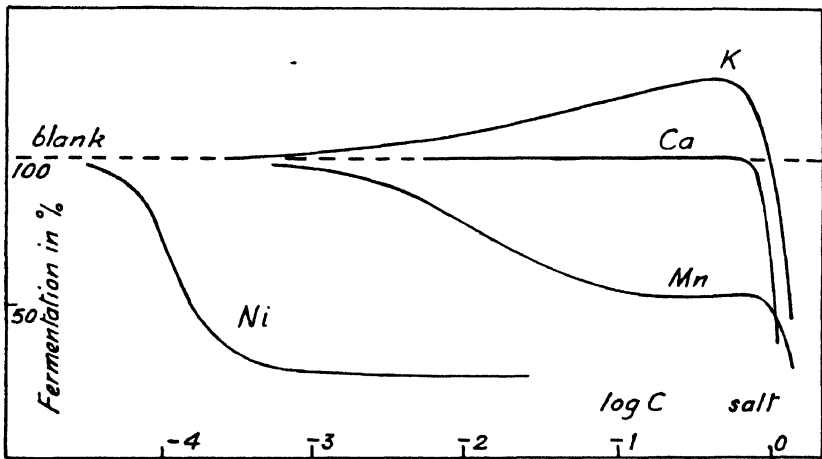


FIG. 7.—Fermentation of sugar by bakers' yeast under the influence of some cations.

fermentation reaches another level and a further addition of the abnormal cation will have no influence. It seems that these experiments show the great importance of Bungenberg de Jong's view on the permeability problem which I call the *complex theory of permeability of Bungenberg de Jong*. This theory has the great advantage that it combines the known factors that play a part in permeability. Only one major question remains to be answered and that is how are we to link the sieve theory and the complex theory? It is therefore necessary to look at a part of the membrane neglected above, the apolar part.

**Apolar Relations in the Membrane.**—First of all one must consider that a large part of the differences in permeability between the various species might find their basis in differences in the apolar chains of the lecithins. It is obvious that the occurrence of double bonds in these chains must influence their mutual distance (and thus the "pore" width) considerably. Here one thinks of the different behaviour of a spread unimolecular layer of stearic acid and that of an acid with one double bond (oleic acid). Bungenberg de Jong and

<sup>23</sup> Booiij, *Rec. trav. botan. Néerl.*, 1940, **37**, 1.

Saubert<sup>15</sup> drew attention to the role played by cholesterol in the membrane: this apolar substance may "condense" the membrane by reason of the London-van der Waals' forces between it and neighbouring lecithin chains. Originally it was thought that the pores of the membrane might be the places not occupied by cholesterol (Fig. 8), but now we realize that this view is too static. In molecular dimensions we must account for the movement of the molecules. Even if an apolar membrane is completely homogeneous one would always find holes in it and the widths of these holes would be distributed statistically. This means that a large polar molecule has practically no chance of slipping through the membrane, while a smaller molecule will find many more opportunities. Permeability laws have to be considered as statistical laws and one is not obliged—as in the original sieve theory—to regard the protoplasmic membrane as a rigid structure with a few large holes, a good many of medium size and many small ones.

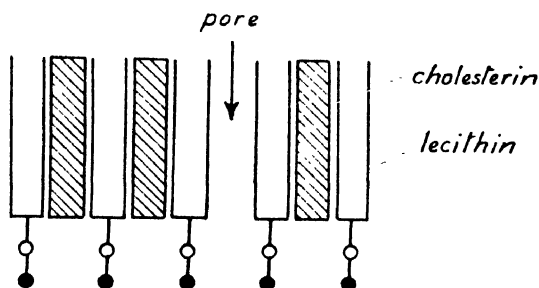


FIG. 8.—The role of cholesterol in the membrane as it was proposed some years ago. This diagram is not "dynamic" enough, as the membrane should not be regarded as a rigid structure.

**Conclusion.**—The complex theory of permeability must be regarded as an attempt to synthesize the many, often conflicting, theories on this subject. According to this view the protoplasmic membrane shows three characteristics:

- (i) it is a "statistical" sieve; (ii) it is a solvent for substances poorly soluble in water; (iii) it is an ion-exchanger.

The possibilities of variations are enormous, which is just what should be expected of an adequate theory of permeability.

(a) The membrane may be uni-, bi- and pauci-molecular.

(b) The arrangement may be such that the lipid molecules point to the aqueous medium, while in other cases the reverse might be expected.

(c) The nature of the lipid (especially the number of double bonds) is of utmost importance.

(d) It might be that the ratio lecithin/phosphatidic acid varies from species to species. That would greatly influence the ionic characteristics of the membrane. Especially the influence of pH will vary considerably. In the biological range of pH the dissociation of the first hydrogen of the phosphoric acid group will be complete, while that of the second hydrogen is very much influenced by a small change of pH. From this it would follow that a membrane with much phosphatidic acid will be very susceptible to pH changes.

(e) The amount of extra apolar substances within the membrane will influence the permeability considerably.

(f) The nature of the protein will vary too from cell to cell; this, presumably, will have a great influence on the ion-exchanging properties. Here there is the possibility that the protein (when it is supposed to lie parallel to the surface of the cell) plays a great part in the elastic properties of the cell's surface.

(g) The cation (and its exchangeability) may vary.

There is a bewildering number of possibilities, which is exactly in accordance with the many divergent facts already known. Some of these are summarized in Fig. 9, in which A shows the structure as it was originally proposed by Bungenberg de Jong and Bonner.<sup>14</sup> Later this was modified B (Bungenberg de Jong and Saubert<sup>15</sup>) as the role of the phosphatidic acids was more clearly understood. One might also suspect that even in a unimolecular layer of lecithin, complex relations are of great importance C. In tricomplex systems with

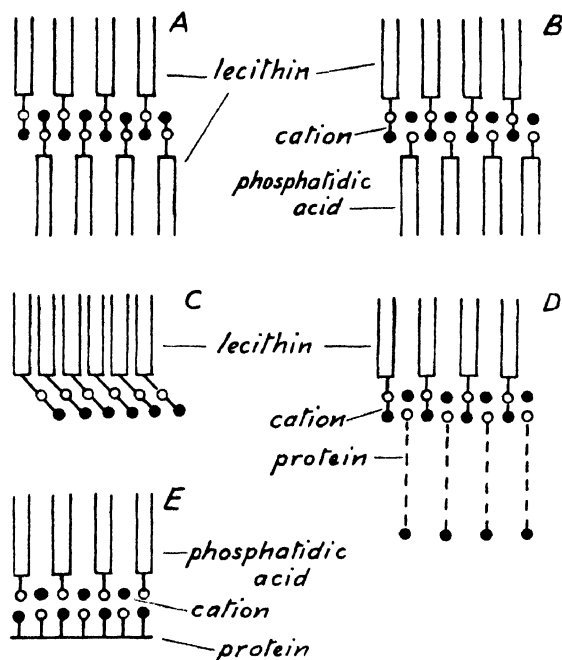


FIG. 9.—A selection of possible membrane structures (more layers might be present and the apolar chains might point to the medium as well as to the cell's interior).

proteins, two extreme cases have been pictured in D and E. In the case of lecithin only the negative groups of protein are of importance for the complex relations, whereas with phosphatidic acid both negative and positive groups play their part.

Now it might be asked, how we are to study permeability problems in future. It is my opinion that investigations on the influence of various substances on permeability will be of the greatest value. Additionally a thorough study of the same substances on systems with parallelly arranged carbon chains must give the basis on which to build our knowledge of the structure of the living membrane. Then experiments with unimolecular layers of lecithin, fatty acids, etc., come to the mind. In our laboratory we are now experimenting on a large

scale on oleate coacervates where the volume of the coacervate layer is greatly influenced by added organic substances. This means that the structure of the orderly built soap micelles (seen as a model for the membrane structure) is changed. So far these experiments seem to give promising results.

When we are interested in the ion-exchange capacities, another type of investigation is useful, namely to measure the reversal of charge of colloids by certain cations. This leads Burgenberg de Jong and his co-workers to the idea that the background of ion-antagonism is essentially a cation-anion antagonism;<sup>24</sup> that  $\text{Ca}^{++}$ - $\text{K}^+$  antagonism is shown very well by phosphate colloids and not by carboxyl and sulphate colloids; and that only lecithin shows a marked resemblance between the influence of ephedrine and  $\text{Ca}^{++}$ , and of acetylcholine and  $\text{K}^+$ .<sup>25</sup> All of these facts strongly suggest that phosphatides play a most important part in many biological phenomena.

Summarizing, it seems advantageous to study the problem of the structure of the protoplasmic membrane along the lines indicated above. We might even find that these investigations have a great value for other biological structures (e.g. protoplasm itself) as the same bio-colloids are found throughout the whole cell.

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<sup>24</sup> Bungenberg de Jong, Booij and Wakkie, *Kolloidchem. Beih.*, 1936, 44, 254.

<sup>25</sup> Teunissen-van Zijp, *Thesis* (Leiden, 1938).

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## LIPID AND PROTEIN COMPONENTS IN THE SURFACE ULTRASTRUCTURE OF THE ERYTHROCYTE

BY ERIC PONDER

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The surface layers of the mammalian red cell are considered as a complex ultrastructure of radially oriented lipid and tangentially oriented protein, the lipid and protein probably being associated rather than spread out in separate layers. The total thickness of the surface ultrastructure is 200-400 Å, some regions being thicker than others. Evidence is considered for believing that the interior of the cell has a certain orderliness, the Hb molecules being oriented as in an expanded crystal, and readily passing into a paracrystalline state. The possible effects of an orderly interior on the surface configuration, and on properties such as the penetration of ions and non-electrolytes, are briefly referred to.

There being no doubt but that the surface layers of cells are regions with special properties, the problem of their structure can be approached in two different ways. The first of these puts emphasis on differential permeability phenomena, and leads us to ask what kind of surface structure can produce the differential barrier effects observed. Attempts to answer this type of question result in classical "membrane permeability theory," which rests on observations of the rates at which many

different substances penetrate into many different cells. The desirable generality of classical permeability theory, however, is off-set by the fact that it tries to account for all permeability phenomena in terms of the properties of the surface layers alone, any influence of the cell interior being largely discounted; the ambiguity of some of the inferences upon which it is based has also led to what Chargaff calls "biological dialectics." The second approach depends on observations made with the microscope, polarization microscope, and electron microscope, together with information obtained by chemical analysis of what are presumed to be intact surface ultrastructures; these observations are more concrete but are more limited, since they have so far been made on a few special types of cell only. One of these is the mammalian erythrocyte, one of several prototypes of the differentially permeable cell; I shall discuss the nature and arrangement of the lipid and protein components of its special surface ultrastructure primarily from the standpoint of chemical analysis and optical measurements, but shall try at the same time to relate the observations to other properties of the cell surface.

**Materials Available.**—It is by no means certain that the surface of the red cell is composed of a structure (a "membrane") which can easily be isolated without loss or contamination. The "fixed framework" or "ghost" prepared by the methods of Erickson and her collaborators,<sup>1</sup> of Fricke, Parker and Ponder,<sup>2</sup> or of Parpart<sup>3</sup> is washed repeatedly, and this may remove enough protein to make up a surface layer at least 20 Å thick, together with enough lipid to make up a similar layer at least 60 Å thick. Again, most preparations of ghosts contain considerable amounts of Hb, possibly together with other substances derived from the cell interior. Lastly, there is still doubt as to how much of the material of the fixed framework makes up the surface ultrastructure and how much is derived from the interior; this doubt will remain until we can decide whether the red cell is a balloon-like body with fluid contents or a body with an organized interior surrounded by still more highly organized surface layers.

We can arrive at tentative conclusions as to the thickness of the surface ultrastructure if we ignore the losses and assume that all the materials found in the fixed framework enter into the surface layers; taking the values for the human red cell, we find a fixed framework which is 3.4 % (dry weight) of the material in the intact cell to give a surface ultrastructure about 190 Å thick. Corresponding values for dog, rabbit, sheep, and ox work out at 250 Å, 125 Å, 120 Å and 75 Å respectively. There is no reason to think that the thickness is the same for all mammals, but since a  $\pm 1$  % variation in the value for the fixed framework corresponds to a 60 Å variation in thickness, reliance should not be placed on small differences. These values do not allow for the contribution of water.

The average quantity of total lipid in the human red cell would make up a continuous surface layer 31 Å thick and of density 0.85 g. cm.<sup>-3</sup>; the corresponding figures for the cells of dog, rabbit, and sheep are 38 Å, 33 Å, and 32 Å. This is about sufficient to form a bimolecular layer. There is not sufficient cholesterol to form even a monolayer, except perhaps in the ox. The ratio of cholesterol to total lipid varies from 1 in 5 in human cells to 1 in 1.5 in ox cells; this is one manifestation of the variability of the lipid composition of the surface ultrastructure from animal to animal, a variability which is also shown by the inconstancy

<sup>1</sup> Erickson, Williams, Bernstein, Arvin, Jones and Macy, *J. Biol. Chem.* 1938, **122**, 515.

<sup>2</sup> Fricke, Parker and Ponder, *J. Cell. Comp. Physiol.*, 1939, **13**, 69.

<sup>3</sup> Parpart, *ibid.*, 1942, **19**, 248.

of the amounts of cholesterol as opposed to cholesterol ester, lecithin as opposed to cephalin, and of "neutral fat", now regarded as a cerebroside fraction (see Tables 4e).<sup>\*</sup> There has been a special interest in the proportions of the lipid fractions because of a hope that differences would account for differences in permeability properties; they do not do so, however, in any simple way (Parpart and Dziemian<sup>5</sup>). The cholesterol molecules are probably arranged at intervals along the lipid palisade; this arrangement would have a stabilizing effect on the otherwise dispersible cephalin (Winkler and Bungenberg de Jong<sup>6</sup>; Dervichian<sup>7</sup>).

The protein found in the human ghost would make up a continuous surface layer 37 Å thick and of density 1.3 g. cm.<sup>-3</sup>. The principal protein, stromatin (Jorpes<sup>8</sup>), can be prepared almost free of Hb, and belongs to a class of proteins similar to, but not identical with, the keratins and collagens (Ballantine<sup>9</sup>). Its molecule is a long structure, exhibiting marked double refraction of flow (Boehm<sup>10</sup>; these results have been questioned by Furchgott<sup>11a</sup>); it may accordingly gel in low concentrations. The quantities found in the ghosts of various mammals vary, but since the amino-acid composition is the same irrespective of source, the immunological differences must depend on small differences in molecular structure. The *A* and *B* factors are associated with polysaccharide bearing side chains, but the *Rh* factor is associated with an ether soluble lipo-protein fraction ("elenin," Calvin, Evans, Behrendt, and Calvin<sup>12</sup>), separable from stromatin at pH 8. In addition to stromatin an unidentified *b*-protein can be separated by electrophoresis (Stern, Reiner, and Silber<sup>13</sup>; their *a*-protein is probably stromatin); a globulin which is eluted during the preparation of stromatin is also present, and intact cells contain a carbohydrate-poor albumin, the "anti-sphering substance," which is readily removed from their surfaces by adsorption on glass (Furchgott,<sup>11a</sup>; Furchgott and Ponder<sup>14</sup>).

The non-ether extractable lipid of the ghost is usually taken as being lipo-protein; using this criterion, from 40 to 60 % of the total lipid is bound to protein (Parpart and Dziemian<sup>5</sup>), probably as secondary valency compounds rather than by salt linkages. Lecithin, with its isoelectric point at pH 6.7, can hardly form salts with proteins at physiological pH's; cephalin and phosphatidyl serine (Folch and Schneider<sup>15</sup>), however, can combine with histone and protamines by ionic bonds, while globin and cephalin form a soluble salt at pH 7. The principal obstacle to the formation of salt-like lipo-proteins seems to be the lack of basic proteins in the red cell ultrastructure.

**Orientation of the Components.**—The evidence bearing on the orientation of the components in the surface ultrastructure is derived from polarization optics, from considerations as to the isoelectric point, and from electron microscopy.

<sup>4</sup> Ponder, (a) *J. Gen. Physiol.*, 1948, **31**, 325; (b) *ibid.*, 1948, **32**, 53; (c) *Haemolytic Phenomena* (Grune and Stratton, New York), 1948.

<sup>\*</sup> Many of the discrepancies in the values for the various lipid fractions are due to different investigators having used different criteria to distinguish between fractions.

<sup>5</sup> Parpart and Dziemian, *Cold Spring Harbor Symposia*, 1940, **8**, 17.

<sup>6</sup> Winkler and Bungenberg de Jong, *Arch. Neer. Physiol.*, 1941, **431**, 467.

<sup>7</sup> Dervichian, *Trans. Faraday Soc.*, 1946, **42B**, 180.

<sup>8</sup> Jorpes, *Biochem. J.*, 1932, **26**, 1488.

<sup>9</sup> Ballantine, *J. Cell. Comp. Physiol.*, 1944, **23**, 21.

<sup>10</sup> Boehm, *Biochem. Z.*, 1935, **282**, 22.

<sup>11</sup> Furchgott, (a) *J. Expt. Biol.*, 1940, **17**, 30; (b) *Cold Spring Harbor Symposia*, 1940, **8**, 224.

<sup>12</sup> Calvin, M., Evans, Behrendt and Calvin, G., *Proc. Soc. Expt. Biol. Med.*, 1946, **61**, 416.

<sup>13</sup> Stern, Reiner and Silber, *J. Biol. Chem.*, 1945, **161**, 731.

<sup>14</sup> Furchgott and Ponder, *J. Expt. Biol.*, 1940, **17**, 117; *J. Gen. Physiol.*, 1941, **24**, 447.

<sup>15</sup> Folch and Schneider, *J. Biol. Chem.*, 1941, **137**, 51.

Rabbit ghosts, prepared by freezing and thawing with subsequent washing in saline, show a faint negative birefringence which is the resultant of the negative form-birefringence of a tangentially-arranged protein framework and the positive birefringence of radially arranged lipid (Schmitt, Bear, and Ponder<sup>16</sup>). The former can be increased by extracting the lipids and by immersing the ghost in mixtures of increasing refractive index. The faint positive birefringence observed in such media could conceivably be produced by a bimolecular layer of lipid situated at the cell surface (Frey-Wyssling<sup>17</sup>) and two observations are often quoted in support of this being the actual arrangement: that of Fricke,<sup>18</sup> whose measurements of the impedance of red cells led to a value of 30 Å for the thickness of a surface film with an assumed dielectric constant of 3, and that of Gorter and Grendel,<sup>19</sup> whose spreading experiments led them to conclude that the acetone-soluble material from the red cells of several species are just sufficient to cover the cell surfaces with a bimolecular layer. The true value of the dielectric constant in Fricke's computations, however, is unknown (it might be much larger than 3, and the surface film correspondingly thicker), while in Gorter and Grendel's experiments the extraction of lipid was almost certainly incomplete. There are, moreover, several objections to the idea that the lipids are arranged at the surface in a *continuous* layer. Some of these are of an immunological nature, e.g. the observation that agglutinins combine predominantly with protein components of the red cell surface and prevent the cells from passing into the oil phase when shaken up with oil-saline mixtures (Mudd and Mudd<sup>20</sup>). Unsensitized cells tend to enter the oil, but if red cells are packed together tightly in haematocrit tubes, they separate without haemolysis when the packed mass is shaken up with saline; there accordingly appears to be no tendency for the surface material of one cell to spread on the surface of its neighbour. To account for observations of this kind, Winkler and Bungenberg de Jong<sup>6</sup> have modified the conception of the surface as covered by a continuous lipid layer by adding an incomplete layer of polar lipids with their hydrophilic groups sticking outwards. The discontinuities in this incomplete layer give the surface a "pore" structure as well as some hydrophilic properties, but the arrangement does not seem to me to have any advantage over one in which both protein and lipid components present themselves at the cell surface.

Further objections to the idea that the surface is covered with a continuous lipid layer arise from measurements of electrical mobility. When these are made at ionic strength 0.172 and with sufficient rapidity to minimize the injurious effects of low pH on the cell surface,\* the isoelectric point of the intact human red cell is at pH 1.7, that of the lipid-extracted ghost at pH 4.7, and that of the extracted and emulsified lipids at pH 2.6.† The pH-mobility curve for cells, lipid-extracted ghosts, and extracted lipids are also of different shape (Furchgott and

<sup>16</sup> Schmitt, Bear and Ponder, *J. Cell. Comp. Physiol.*, 1936, **9**, 89; *ibid.*, 1938, **11**, 309.

<sup>17</sup> Frey-Wyssling, *Submicroscopic Morphology of Protoplasm and its Derivatives* (Elsevier Pub. Co., New York, 1948), p. 171.

<sup>18</sup> Fricke, *J. Gen. Physiol.*, 1925, **9**, 137.

<sup>19</sup> Gorter and Grendel, *J. Expt. Med.*, 1925, **41**, 439.

<sup>20</sup> Mudd, S., and Mudd, E. B. H., *ibid.*, 1926; 1926, **43**, 127.

\* Failure to avoid or to allow for these injurious effects has led several observers to conclude that the isoelectric point of the intact cell is higher than it really is. Some methods of preparing ghosts (e.g. Parpart's CO<sub>2</sub> method) are known to result in surface injury and in reduced mobilities. It is remarkable that at pH 7 identical mobilities are obtained for intact red cells (rabbit), cells lysed by water, and cells lysed by saponin (Abramson, Furchgott, and Ponder<sup>20</sup>).

† There may have been contamination of the surfaces of the lipid droplets with protein.



Ponder <sup>14</sup>). The surface of the intact cell is accordingly neither a protein surface not yet the same as that of lipid droplets extracted from it. The low iso-electric point for intact cells and the flat maximum mobility level above pH 7 suggest that the surface is dominated by strongly acidic groups such as those in cephalin; indeed, still more strongly acidic groups, such as those in the heparins, may be required to account for the data. The data, however, do not exclude the presence in the surface of small amounts of protein, and it should be remembered that the "surface," from the standpoint of electrophoretic mobility measurements, is not necessarily the same as the morphological surface.

The micrographs obtained with the electron microscope point still more convincingly to the red cell surface not being a homogeneous film. Ghosts appear as folded structures with a dry thickness of 250-500 Å (Zwickau <sup>21</sup>; Wolpers <sup>22</sup>; Wolpers and Zwickau <sup>23</sup>; Jung <sup>24</sup>; Rebuck and Woods <sup>25</sup>); after extraction of the lipids, the sheets appear porous. Wolpers <sup>22</sup> has described the surface as having a fibrous appearance, and suggests that the lipids are imbedded as lens-shaped bodies in the meshes of a protein frame-structure. In such an arrangement, there would be a discontinuous layer (or layers) of lipid with radially oriented molecules, the lipid areas being imbedded in tangentially arranged protein fibres; the polarization optics of such a structure would be similar to those observed, and, regarded from the standpoint of penetrating substances, the structure could properly be referred to as a protein-lipid mosaic. The low tension at the surface of erythrocytes (Norris, <sup>26</sup> nucleated red cells of *Triturus*), like that at the surface of the marine eggs (Harvey <sup>27</sup>; Cole <sup>28</sup>), is probably due to the presence of proteins (Danielli and Harvey <sup>29</sup>; Danielli <sup>30</sup>). Whether these proteins are part of the structure, adsorbed, or both, is a question which has arisen in connection with the antisphering substance, and one which cannot be answered with certainty. Any elasticity possessed by the surface ultrastructure (Seifriz <sup>31</sup>; Beams <sup>32</sup>) is best attributed to the "junctions" in the protein framework (Frey-Wyssling <sup>17</sup>).

**Thickness.**—The lipids and proteins found in the human red cell surface ultrastructure would make up a layer about 70 Å thick, but losses during washing and incomplete extraction of lipid tend to make this estimate too low. The estimate, moreover, is based on dry weights, and the real thickness of the structure will depend on the amount of water it contains. Direct measurements with the leptoscope (Waugh and Schmitt <sup>33</sup>) give 220 Å as the maximum thickness in rabbit ghosts; this value can be halved by extracting the lipids, some of which, however, had probably been lost during the preparation of the material. In the rabbit, the protein and lipid would make up a dry layer about 120 Å thick; the contribution of water would therefore seem to be about 80 %, although Waugh and Schmitt prefer a smaller figure (25 %). Some of this water lies between the tangentially arranged fibres of the stromatin, and some of it between groups of molecules of the lipid palisade; such

<sup>21</sup> Zwickau, *Dissertation* (Lab. Übermikroskopie Siemens and Halske, Berlin, 1941).

<sup>22</sup> Wolpers, *Naturwissenschaft.*, 1941, **286**, 461.

<sup>23</sup> Wolpers and Zwickau, *Folia hematologica*, 1942, **66**, 211.

<sup>24</sup> Jung, *Klin. Wochenschr.*, 1942, **21**, 917; *ibid.*, 1947, **29**, 459.

<sup>25</sup> Rebuck and Woods, *Blood*, 1948, **3**, 175; Rebuck, Woods and Monogham, *Proc. Soc. Expt. Biol. Med.*, 1948, **68**, 220.

<sup>26</sup> Norris, *J. Cell. Comp. Physiol.*, 1939, **14**, 117.

<sup>27</sup> Harvey, *Biol. Bull.*, 1931, **60**, 67; **61**, 273.

<sup>28</sup> Cole, *J. Cell. Comp. Physiol.*, 1932, **1**, 1.

<sup>29</sup> Danielli, and Harvey, *ibid.*, 1934, **5**, 483.

<sup>30</sup> Danielli, *Cold Spring Harbor Symposia*, 1938, **6**, 190.

<sup>31</sup> Seifriz, *Protoplasma*, 1927, **1**, 345; *Trans. Faraday Soc.*, 1946, **42B**, 259.

<sup>32</sup> Beams, *Proc. Soc. Expt. Biol. Med.*, 1947, **66**, 373.

<sup>33</sup> Waugh and Schmitt, *Cold Spring Harbor Symposia*, 1940, **8**, 233.

a separation of the surface molecules seems to be necessary in order to allow of reversible disc-sphere transformations in which the surface decreases some 30 % without appreciable volume change (see Ponder <sup>4</sup>), and the intercalated water may itself be oriented as it is in myelin forms\* (Dervichian <sup>7</sup>).

The electron microscope gives even greater thicknesses. Zwickau's estimate is 250-300 Å and Rebeck's (private communication) is about 500 Å; if a 25 % contribution of water is allowed for, the total thickness of the ultrastructure becomes 300-600 Å. The discrepancy between this and the thickness computed from the sum of the lipid and protein may be due to the loss or under-estimation of the latter, or may be due to the surface layers having indefinite boundaries on the side directed towards the cell interior, so that more or less of an organized internal structure comes to be included when different methods are applied to the elucidation of structure. In the "tripartite model" of Winkler and Bungenberg de Jong, for example, the surface stromatin is represented as linked to the outermost layers of the Hb of the interior, and there is also a possibility of cephalin-globin linkages.

To add to the complexity, the thickness and structural composition is neither uniform nor constant. The leptoscope shows the region of the biconcavities to be about 30 Å thicker than the regions near the rim; the difference increases after extraction of the lipids, and so is probably due to the protein components being relatively greater over the biconcavities. In the reversal of the disc-sphere transformation, the biconcavities and even the larger crenations reappear at the same parts of the surface as they occupied before the shape change; this demonstrates a non-uniformity of the surface. Many lysins in low concentrations seem to break down the surface at spots (see Ponder <sup>4</sup> for a discussion of the involved subject of spot-wise lysis), and the amount of Cu required to affect the penetration of glycerol is so small that it must be thought of as acting on groups (possibly sulphhydryl) spaced at wide intervals on the surface (Jacobs and Corson <sup>34</sup>; Jacobs <sup>35</sup>; LeFevre <sup>36</sup>). Finally, the anti-sphering substances can be dislodged from the surface, at least partially, with the result that the discoidal cell becomes a sphere without any detectable change in its permeability properties, and Curtis <sup>37</sup> has shown this shape change is accompanied by alterations in the complex impedance which can be interpreted as arising from a slight thinning of the surface layers. The opposite effect is observed when the cells are placed in hypotonic media, in which they swell and in which their surfaces are distorted and perhaps stretched; Curtis suggests that under these circumstances new material comes from the cell interior to supplement the surface layers. A similar result has been obtained for the egg of *Hippionoe* (Cole <sup>38</sup>).

**The Interior.**—Evidence is accumulating that the red cell interior has a certain orderliness. The concentration of Hb is so high that the average distance between molecules is 75 Å; this figure corresponds to the diameter of the smallest sphere in which the cylindrical Hb molecule

\* It may be helpful to think of the structure of the surface in the same terms as Dervichian uses when speaking of myelin forms, i.e. in terms of a balance between the free energy of molecular cohesion and the free energy of affinity for water. Myelin forms are produced from the red cell ultrastructure under several conditions (Auer, <sup>31</sup>; Furchgott, <sup>11b</sup>), and the intact surface may be thought of as a myelin form in which the extension is particularly small because of the superadded cohesive effects of parallel and underlying protein linkages ("hindered solubility effects").

<sup>34</sup> Jacobs and Corson, *Biol. Bull.*, 1934, **57**, 325.

<sup>35</sup> Jacobs, *ibid.*, 1946, **91**, 237.

<sup>36</sup> LeFevre, *ibid.*, 1947, **93**, 224; *J. Gen. Physiol.*, 1948, **31**, 505.

<sup>37</sup> Curtis, *ibid.*, 1936, **19**, 929.

<sup>38</sup> Cole, *ibid.*, 1928, **12**, 29.

is free to rotate\* (Perutz<sup>39</sup>), and to the X-ray diffraction maxima observed for red cells by Dervichian, Fournet and Guinier.<sup>40</sup> An acceptable picture is accordingly one of freely rotating Hb molecules in a close-packed lattice, i.e. of an arrangement (an "expanded crystal") in which there is short-range order intermediate between the three-dimensional order of a crystal and complete disorder. The interpretation of X-ray and birefringence data, however, is complicated by the possibility that the internal structure, like the surface ultrastructure, may be heterogeneous although essentially orderly; effects from one region may cancel those from another, and so the degree of orderliness would tend to be underestimated. Other types of evidence point to the molecules of the interior being arranged in an orderly way (possibly in lamellae, Teitel-Bernard<sup>41</sup>): the failure of intracellular Hb to crystallize, although crystallization may occur in smaller concentrations after haemolysis (Roepke and Baldes<sup>42</sup>), the sudden increase in metabolism accompanying lysis (Ramsey and Warren<sup>43</sup>), and the birefringence which occurs when a small amount of water has been abstracted from the cell (Teitel-Bernard<sup>41</sup>), and sometimes even spontaneously (Ponder<sup>44</sup>).

The question of the orderly arrangement of the interior is further complicated by the possibility of its containing substances other than Hb. The red cells of both the rat (Beams and Hines<sup>45</sup>) and man (Beams<sup>32</sup>) can be stratified in the ultracentrifuge into three layers, one of Hb, one of lipid, and one of a colourless component which may be a matrix substance. Again, the almost completely reversible formation of the sickle cell is accompanied by a withdrawal of its Hb into what corresponds to the rim of the cell, leaving a fringe of colourless material in the concavity of the sickle;† the haemoglobinized part later becomes strongly birefringent (Sherman<sup>46</sup>; Itano and Pauling<sup>47</sup>). The objection usually made to there being a matrix substance in addition to Hb is that there is not sufficient protein and lipid left in the ghost to form an internal framework when the components required for the surface layers have been allowed for. Material other than Hb however, may leave the cell when it haemolyses and so may not be recovered in the ghost; this possibility has not been inquired into carefully.

**Possible Effects of an Orderly Interior on Surface Configuration and Properties.**—The existence of an orderly arrangement in the red cell interior would influence our ideas as to the structure and properties

\* Normal red cells are not birefringent although individual Hb molecules are anisotropic, and on these grounds it is likely that the Hb molecules are free to rotate. Perutz has called attention to the kinetics of the reaction between CO and HbO<sub>2</sub> being the same in the red cell as it is in solution (Roughton<sup>48</sup>). A large increase in affinity for O<sub>2</sub>, however, although not produced by the mere lysis of the red cell without dilution of its contents, becomes apparent when dilute cell suspensions are compared with dilute Hb solutions (Hill and Wolvenkamp,<sup>49</sup>). This effect, the nature of which is not clear, is not due to pH and differs in different mammals.

<sup>39</sup> Perutz, *Nature*, 1948, 161, 204.

<sup>40</sup> Dervichian, Fournet and Guinier, *Compt. rend.*, 1947, 224, 1948.

<sup>41</sup> Teitel-Bernard, *Arch. Roumain. Path.*, 1932, 5, 389.

<sup>42</sup> Roepke and Baldes, *J. Cell. Comp. Physiol.*, 1942, 20, 71.

<sup>43</sup> Ramsey and Warren, *Quart. J. Expt. Physiol.*, 1934, 24, 153.

<sup>44</sup> Ponder, *J. Gen. Physiol.*, 1945, 29, 89.

<sup>45</sup> Beams and Hines, *Anat. Record.*, 1944, 90, 155.

† Rebeck's electron micrographs of sickle cell filaments show a dense material in the part of the filament near the cell, and also a periodic distribution of a dense material more distally. The width of the filament is only 1000-3000 Å and its length several μ; if the dense material is Hb, its association with lipid in a structure so thin and extended is surely a molecular association and not just the enclosing of Hb in a phospholipid tube. Dr. Rebeck has kindly let me see some of his unpublished material.

<sup>46</sup> Sherman, *Bull. Johns Hopkins Hosp.*, 1940, 67, 309.

<sup>47</sup> Itano and Pauling, *Blood*, 1949, 4, 66.

of the surface layers in several ways. The customary distinction between surface and interior would lose its sharpness, and would be replaced by an arrangement, such as that in Winkler and Bungenberg de Jong's model, in which the lipids and proteins of the surface layers are united to the underlying Hb so as to make up a structure extending throughout the cell thickness, although perhaps with decreasing orderliness from without inwards. In such a structure, the form and spatial arrangement of the Hb molecules and the orientations in the surface might be integrally related to each other, the former even acting as a kind of template for the latter. The lysis of such a structure would then involve more than effects on its surface layers, and Jonxis'<sup>48</sup> observation that cells containing fetal Hb are preferentially affected by the *Rh* agglutinin-lysin would become understandable \*; similarly, a special composition of the Hb of the sickle cell may underlie the occurrence of the sickling phenomenon (cf. Watson<sup>49</sup> whose results suggest that cells do not sickle if they contain fetal Hb). A number of properties of the red cell, e.g. crenation and the assumption of paracrystalline states (Ponder<sup>4</sup>), the fragmentation and shape changes resulting from heat (Ponder<sup>50a,b</sup>), stratification, the sickling phenomenon and the fine structure of sickle filaments, some of the phenomena described by Auer,<sup>51</sup> etc., can be accounted for more readily on the basis of there being an orderly structure throughout its thickness than on the basis of surface layers enclosing a Hb-containing fluid.

It cannot be expected that the penetration of substances into a cell with such a complex surface ultrastructure, and possibly with an expanded-crystal type of interior as well, will be adequately accounted for by classical membrane permeability theory. The conditions which govern the passage of material across an anion-permeable, but cation- and Hb-impermeable, membrane separating two dilute solutions have been elegantly treated by this theory; much of the behaviour predicted for a model system of this kind can be observed when the red cell is substituted as the experimental object, but in other respects the model and the object behave differently. The cell swells in hypotonic media as an osmometer of very varying degrees of imperfection; the cell is not cation impermeable as the model is, and the slow penetration of cations observed, even if provided for by investing the model with a membrane through which cations move slowly, is not followed by the indefinitely great swelling which the theory calls for (Ponder<sup>4a,b</sup>). A process of cation exchange, together with a limited amount of swelling, occurs instead, and this process appears, moreover, to be dependent on metabolism (Wilbrandt<sup>52</sup>; Harris<sup>53</sup>; Davson and Reiner<sup>54</sup>; Ponder<sup>50c</sup>). What seems to be required to account for the behaviour of the cell towards penetrating substances is a surface mosaic in Brooks'<sup>55</sup> special sense of the term, i.e., a structure in

\* This, however, is an observation which I cannot confirm, since my own determinations show the same proportion of fetal Hb in the cord blood of normal infants as in the cord blood of infants affected with hemolytic disease. Baar<sup>56</sup> has also pointed out that Jonxis' conclusions are not at all compatible with results obtained by him and his collaborators.

<sup>48</sup> Jonxis, *Nature*, 1948, **616**, 850.

<sup>49</sup> Watson, *Amer. J. Med. Sci.*, 1948, **215**, 419.

<sup>50</sup> Ponder, (a) *J. Gen. Physiol.*, 1949, **32**, 399; (b) *J. Expt. Biol.* (in press); (c) *J. Gen. Physiol.* (in press).

<sup>51</sup> Auer, *J. Expt. Med.*, 1932, **56**, 551.

<sup>52</sup> Wilbrandt, *Trans. Faraday Soc.*, 1937, **33**, 956.

<sup>53</sup> Harris, *J. Biol. Chem.*, 1941, **141**, 579.

<sup>54</sup> Davson and Reiner, *J. Cell. Comp. Physiol.*, 1942, **20**, 325.

<sup>55</sup> Brooks, *Trans. Faraday Soc.*, 1937, **33**, 1002.

<sup>56</sup> Abramson, Furchgott and Ponder, *J. Gen. Physiol.*, 1939, **22**, 545.

<sup>57</sup> Roughton, *Amer. J. Physiol.*, 1945, **143**, 609.

<sup>58</sup> Hill and Wolvenkamp, *Proc. Roy. Soc. B*, 1936, **120**, 484.

<sup>59</sup> Baar, *Nature*, 1948, **162**, 190, 1948.

which there are pathways for anions side by side, on a molecular scale, with pathways for cations, in which the exchange of cations and perhaps of some non-electrolytes (glycerol and sugars) is controlled by metabolic processes, and in which there are additional pathways along which lipid-soluble substances can move. It is not likely that an adequate theory of penetration can be developed quantitatively until more is known about the state of the interior, the activity of ions in it, and the way in which the metabolic processes produce their spatially directed effects; the complex behaviour, however, no doubt has its origin in the elaborate nature of the architecture.

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## THE CONTROL OF THE BODY TEMPERATURE BY FATTY ACID MONOLAYERS

By J. M. O'CONNOR

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The standard conception of temperature regulation is that it depends upon a control of heat production and of heat loss exercised by centres in the brain. But as these centres may be cut off from the mass of the body without any essential loss of temperature maintenance, their significance must be subsidiary in character. Re-examination of the influence of temperature on the oxygen consumption of cold blood and warm blood animals and of tissue preparations from them shows that the increase with rising temperature is not, as was believed, continuous, but exhibits falls about 30° C and about 36° C. The fact that in the neighbourhood of 36° the heat production rises with falling temperature must be a substantial and fundamental element in the fixation of the body temperature in this temperature zone. As an explanation of these two falls it has been suggested that oxidative metabolism is dependent upon the presence of an unimolecular layer of fatty acids covering a surface (possibly the surface of an oxidizing catalyst) and that the falls at 30° and 36° are due to solution out of the monolayer of oleic and palmitic acid respectively. Evidence is given in proof of this view. Physical experiments are alluded to which show that a monolayer of palmitic acid *in vitro* behaves in a fashion in approximate agreement with the requirements of the theory.

The purpose of this communication is to show that the constancy of the body temperature in warm-blooded animals, which is commonly believed to be superimposed upon a primitive production of heat through the activity of a special central co-ordinating mechanism, is in reality a consequence of the nature of metabolic oxidation. Arguments will be given to show that the intensity of oxidation depends upon the area of a unimolecular layer of fatty acid the size of which varies with temperature in such a fashion that the body temperature is held at a level determined by a physical property of the layer.

A constancy of body temperature is maintained so long as heat production and heat loss balance. It has long been accepted that this balance is actively supervised by a thermostatic centre in the brain. The essential argument for this is that the temperature falls when the spinal cord is cut. Closer inquiry has revealed that a region of the

hypothalamus exercises a control over heat loss. It has been shown that when this region is damaged the body temperature becomes less stable and that, when this region is heated, mechanisms for the dissipation of heat are put into action. It has also been suggested that this or a neighbouring region is also responsible for the increase in heat production which appears on exposure to low temperatures, but this has not been established.

The occurrence of an increase in heat production on exposure to cold was noticed by Crawford and by Lavoisier, but an active interest in the nature of the increase dates from the investigations of Liebermeister. Consequent on his work the relation between body temperature and heat production was actively studied by Pflüger and his pupils, who, sceptical of the suggestion that cooling of the tissues caused an increase in heat production, produced evidence purporting to show that in cold-blooded animals the rate of oxidation rises steadily with increasing temperature, and that the same rule holds for warm-blooded animals provided that muscular movements, in particular shivering, are prevented. Since that time it has come to be accepted that the increase in heat production on exposure to cold results from a central stimulation of muscular metabolism.

The whole basis of this conception of temperature control fails when it appears that after section of the spinal cord the animal in a few days regains and maintains its normal temperature. This was established very clearly by Goltz and Ewald who showed that when as much of cord as could possibly be dispensed with was removed the dog regained temperature control. This long neglected observation was again established by Popoff, by Thauer and by others. A discussion of these matters is given by Thauer<sup>1</sup> and also by O'Connor.<sup>2</sup> In the absence of any other conceivable co-ordinating system it would seem that the constancy of the body temperature must be inherent in the body itself and does not depend on any superimposed co-ordination.

A systematic re-investigation of the influence of temperature on the heat production of various animals and animal preparations showed that so far from being, as Pflüger and those who followed him believed (cf. Krogh<sup>3</sup>), a continuous increase with rising temperature, there are two sharp decreases, one at or above 30° and a second at the normal body temperature. A search for these changes was instigated by a study of the relations between temperature, shivering and the sensation of cold (O'Connor<sup>4</sup>). It was shown first that falls in oxygen consumption occurred at about 30° and 36° in the anaesthetized curarized rabbit (O'Connor *et al.*,<sup>5</sup> O'Connor<sup>6</sup>). The occurrence of these and other minor departures from uniformity have been established subsequently in a variety of cases. The frog shows a sharp though small increase at about 15° and a marked fall at 32° (O'Connor and O'Donovan<sup>7</sup>). The earthworm shows, in addition to these two, a fall at 17° (O'Connor<sup>8</sup>). As frogs and earthworms do not commonly survive 32° it might be thought that a fall at the point of death is not remarkable and should

<sup>1</sup> Thauer, *Erg. Physiol.*, 1938, **41**, 607.

<sup>2</sup> O'Connor, *Irish J. Med. Sci.*, 1943.

<sup>3</sup> Krogh, *The Respiratory Exchange of Animals and Man* (Longmans, London, 1916).

<sup>4</sup> O'Connor, *Proc. Roy. Irish Acad. B*, 1932, **40**, 175.

<sup>5</sup> O'Connor, Moriarty and FitzGerald, *ibid.*, 1935, **42**, 345.

<sup>6</sup> O'Connor, *ibid.*, 1936, **43**, 23.

<sup>7</sup> O'Connor and O'Donovan, *ibid.*, 1942, **47**, 251.

<sup>8</sup> O'Connor, *ibid.*, 1942, **48**, 85.

not be stressed, but when one finds in the observations of Benedict<sup>9</sup> that tropical cold-blooded animals, which tolerate higher temperatures, also show a fall at 32° it seems not unlikely that this fall in frog and earthworm is the cause rather than the consequence of the collapse. Although tropical "cold-blooded" animals tolerate a temperature of 35° they rarely survive at mammalian temperatures, and investigation in this range must be done on mammals or birds and in them only when the confusion produced by muscular movements has been suppressed by curare or some other measure. It has been already mentioned that the falls at 30° and at 36° occur in anaesthetized curarized rabbits. The fall at normal body temperature was also demonstrated in unanaesthetized rabbits in a collection of data from the literature (O'Connor<sup>10</sup>) but among these there was not an adequate number to show that a fall occurs also at the lower point. The fall at 36° was also demonstrated in the observations presented by Freund and Janssen<sup>11</sup> on the relation between temperature and oxygen consumption of the denervated gastrocnemius muscle of the cat. These authors endeavoured to use their results as an argument for the passage of nerve impulses, influencing heat production, from the cut spinal cord along the arterial wall, but the facts are completely explained as a direct consequence of the temperature of the muscle itself (O'Connor<sup>10</sup>).

A very convenient method of demonstrating the pattern of the relation between temperature and oxygen consumption can be used on the human skin. The reciprocal of the time taken, after occlusion of the circulation in the finger, for the  $\alpha$  absorption band of oxy-haemoglobin to disappear from light reflected from it gives a measure of the intensity of oxidation (Vierordt and Dennig). When an adequate number of observations made at different temperatures is plotted, decreases at 32° and 36° are obvious (O'Connor<sup>12</sup>).

It is clear that the fall in oxygen consumption at 36° found in so many mammalian preparations must have significance in connection with the maintenance of the body temperature, but it appears reasonable and in accordance with established conceptions to suggest that the change is imposed by some regulating mechanism. It is true that it is difficult to imagine how such a mechanism could cause a response, as in the last example, in a local area of the skin in which alone the temperature has altered. It is also true that the occurrence of a comparable fall at 32° in both warm and cold-blooded animals, to which no immediate functional significance can be attached, gives ground for doubting that the change at the higher temperature can be considered to be essentially different. But neither of these arguments removes the need for proving that the changes are inherent in the tissues by showing that they occur in isolated tissue. That this is so has been shown for the skin of the frog. There is a fall at 31° and a very marked fall in level between 35.5° and 43°—which is the range within which the temperatures of warm-blooded animals fall with the exception of the monotremes among which it occurs at about the level of the lower fall (O'Connor<sup>13</sup>). This demonstration is open to the criticism that the frog tissue was necessarily exposed to temperatures widely outside

<sup>9</sup> Benedict, *The Physiology of Large Reptiles* (Carnegie Inst., Washington, 1932).

<sup>10</sup> O'Connor, *Proc. Roy. Irish Acad. B*, 1936, 43, 34.

<sup>11</sup> Freund and Janssen, *Pflüger Arch.*, 1923, 200, 96.

<sup>12</sup> O'Connor, *Proc. Roy. Irish Acad. B*, 1938, 44, 129.

those which it normally experiences or tolerates. Experiments were consequently done on the ox cornea which eventually gave the expected result. But a discussion of them had best be postponed until a theory accounting for the peculiar relation between temperature and oxygen consumption has been advanced.

Before turning to this section of the argument the question must be faced why these falls which appear so distinctly and so readily had not been previously noticed. The fall at  $30^{\circ}$  has not been noticed because observations below this level were made practically only on cold-blooded animals and were not continued above it until Benedict's observations on tropical animals. As observations on warm-blooded animals require curare or cord section they are comparatively few and the number in the neighbourhood of  $30^{\circ}$  is almost negligible. The important fall at  $36^{\circ}$  has not been observed because the matter was regarded as having been decided by Pflüger. A critical examination of Pflüger's data shows that his conclusion was unjustified and that in a combination of his data and those of his pupil Velten the fall with rising temperature within the range of the normal rabbit temperature is clearly visible (O'Connor<sup>13</sup>).

If these falls be not imposed from outside the cells they must be caused by some change in the oxidative system. According to the Arrhenius equation the rate of oxidation in a system should rise with rising temperature. If there be a fall it must be due to decrease in the substrate or diminution in the activity of the catalyst. As the falls appear systematically at, or in the neighbourhood of, the same temperatures, physical changes in state in a body constituent at these points were sought and attention was attracted by the properties of monolayers of fatty acid (O'Connor, 1938). Numerous parallels were found. Palmitic acid could not, it seemed, easily form a monolayer above  $36^{\circ}$ . If a monolayer of fatty acid were necessary for, and its area determined the intensity of the activity of a catalyst, the disappearance of palmitic acid out of the monolayer would explain the important fall at  $36^{\circ}$ . That the palmitic acid begins to fade out of a monolayer at about  $36^{\circ}$  was confirmed by Kane (personal communication). The sudden increase at  $15^{\circ}$  noted in frogs and worms coincided with the entrance of palmitic acid into a monolayer (under 6 dynes pressure) at this temperature (Cary and Rideal<sup>14</sup>). The fall at  $30^{\circ}$  corresponds to the disappearance of oleic acid out of a monolayer. Striking was a coincidence involving lauric acid. This acid cannot form a monolayer above  $17^{\circ}$ . It is not a common constituent of natural fat. It occurs in the earthworm to the extent of 5%. The earthworm, as mentioned previously, shows a fall in oxygen consumption at  $17^{\circ}$ . There is no such fall in the frog which has no lauric acid.

These coincidences made the theory plausible and an experimental proof was sought. If it were possible to alter the proportions of the fatty acids occurring in a particular tissue, the extent of the falls in the rate of oxidation occurring at the two crucial points and the level of the metabolism at different temperatures should also alter. In fact it was found that dressing the skin of the finger for several days before examination with different normally occurring fatty acids produced the theoretically expected alterations in the pattern of the effect

<sup>13</sup> O'Connor, *Proc. Roy. Irish Acad. B*, 1947, 51, 211.

<sup>14</sup> Cary and Rideal, *Proc. Roy. Soc. A*, 109, 125, 301.



of temperature. Lauric, myristic, palmitic, stearic and oleic acid were examined. The results with oleic acid were particularly striking: the fall at  $32^{\circ}$  was much more pronounced and the fall at  $36^{\circ}$  much less (O'Connor<sup>15</sup>). Recently a striking result was obtained with margaric acid.<sup>16</sup> If the fall at  $36^{\circ}$  is due to the disappearance of palmitic acid out of the monolayer and the final fall at  $44^{\circ}$  to the disappearance of stearic acid, the introduction of the intermediate (margaric) acid, which does not occur in natural fat, should result in a new fall appearing midway between these two points. When the finger skin had been previously dressed with margaric acid a new marked fall appeared at  $40^{\circ}$ .

The experiments on the influence of temperature on ox cornea (O'Connor and McKeever<sup>17</sup>) may now be examined. An examination of a preparation of isolated mammalian tissue was particularly desirable because Field *et al.*<sup>18</sup> in an investigation of mammalian nervous tissue had not found evidence for the fall at  $32^{\circ}$ . The results with the cornea showed falls at  $34^{\circ}$  and  $42^{\circ}$ . Neither of these could account for the maintenance of the ox temperature at the normal ( $36.7^{\circ}$ - $39.1^{\circ}$ ). But the fall at  $42^{\circ}$  would correspond with the maintenance of a febrile temperature. For if the normal temperature be dependent on a tissue phenomenon the pyrogens responsible for fever must also act peripherally causing a shifting upwards of the fall at  $36^{\circ}$ . If this is true antipyretics should produce their effect on tissues by bringing the raised point of fall in heat production back to normal. It was found that treatment of the corneae with 0.0015 % sodium salicylate in Ringer's solution for a quarter of an hour before investigating the oxygen consumption caused the falls at  $34^{\circ}$  and  $42^{\circ}$  to be replaced by falls at  $33^{\circ}$  and  $37^{\circ}$ . The extent of each of these falls is in approximate agreement with the proportions of oleic, palmitic and stearic acid in ox fat.

Three questions must be alluded to in conclusion. The upper limits of the stability of fatty acid layers have been somewhat cursorily dealt with in the past. Recently Mr. Armstrong (working for the Medical Research Council of Ireland) has shown that the area covered by palmitic acid on N/100 acid begins to decrease at  $36^{\circ}$ . On a buffer solution at pH 6.5 the rate of disappearance is very much more marked. The temperature at which this fall begins on this solution is more variable and usually somewhat higher. The cause of this variation is being investigated.

A second question is, on what surface do these fatty layers form? On this there is no information. It is plausible that the available amount of free fatty acid capable of contributing to the monolayer determines the state of aggregation of an oxidative catalyst, of a protein nature, which determines the rate of oxidation, but there is no direct evidence in support of this surmise.

Finally, it must be noted that there are two sides to the balance of heat and only one has been considered. Heat loss is in the main controlled by the flow of blood to the skin. This flow is normally affected by central and reflex impulses but that these can be dispensed with has been shown in the animals in which the spinal cord has been destroyed. The loss of heat must then be controlled by the local effects of temperature on the skin vessels.

<sup>15</sup> O'Connor, *Proc. Roy. Irish Acad. B*, 1942, 48, 93.

<sup>16</sup> O'Connor, *ibid.* (in press).

<sup>17</sup> O'Connor and McKeever, *ibid.* (in press).

<sup>18</sup> Field, Fuhrman and Martin, *J. Neurophysiol.*, 1944, 7, 117.

It is known that surviving arteries dilate on warming (Cruickshank and Rau<sup>19</sup>). In conjunction with Mr. J. Edozien the relation between temperature and tone is being re-investigated. The results obtained so far with the carotid artery of the ox seem to bear the interpretation that here also the fatty acids have significance. It is true that the theory of tone of involuntary muscle is uncertain and possibly complicated, but the changes which occur on warming show simple parallels with the changes in properties of fatty acid monolayers. The most striking contraction on heating from a low temperature occurs at 16° thus corresponding with the entrance of palmitic acid onto a surface in a close-packed or cohering form, and the most pronounced relaxation takes place over the temperature ranges at which palmitic and stearic acid undergo expansion. Indeed it appears that the whole course of the effect of warming can be represented by calculating the difference at each temperature between the area which would be occupied by a fixed number of molecules according to the gas laws and the area which would be occupied by fatty acids molecules in accordance with their properties and the proportions in which they occur in the fat of the ox. If this view can be sustained it will appear that the bond which holds the temperature of the body constant is the physical properties of its simplest organic constituent.

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<sup>19</sup> Cruickshank and Rau, *J. Physiol.*, 1927, 64, 65.

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#### GENERAL DISCUSSION \*

**Dr. H. L. Boolj** (*Leiden*) said: One should realize that carrageen is much more hydrophilic than cetyl sulphate, so experiments on cetyl sulphate must not be compared directly with those on carrageen. There seems to be little evidence for the idea that there is an invisible precipitate of carrageen in low concentrations of  $\text{CaCl}_2$ , as there is no Tyndall effect and as the reversal of charge is reached only at very high concentration. Moreover, the tri-complex between lecithin, carrageen and  $\text{Ca}^{++}$  appears practically only between the points of reversal of charge of the two colloids involved.

**Dr. A. Lasnitzki** (*Birmingham*) said: May I comment on a point in the paragraph "Electrical Relations in the Protoplasmic Membrane" of Dr. Boolj's paper. I think that it is difficult to employ, without further analysis, the effect of cations upon a metabolic process—in this particular case fermentation of sugar by yeast—as a reliable indicator of possible changes in cell permeability produced by them through structural alterations in the protoplasmic membrane. Evidently, the underlying idea is that the cations merely control the inward diffusion of the substrate from the medium into the cell, but do not exercise any appreciable influence on the enzyme systems responsible for the metabolic process in the cell interior. This supposition is, however, not in agreement with quite a number of results in which the direct (stimulating or inhibiting) action of cations upon various enzymes, present in intact cells as well as cell extracts, could be demonstrated.

**Dr. H. L. Boolj** (*Leiden*) said: I am very thankful for Dr. Lasnitzki's

\* On three preceding papers.

remark, as it enables me to point out why we think that some inorganic cations change cell permeability and that they do not act on enzyme systems within the yeast cell.

(i) It is a well-known fact that the rate of permeation of many inorganic cations into protoplasm is very low. Supposing that these cations act on one or more enzyme systems, one would expect that the fermentation would be affected after a certain lapse of time only. The experiments show, however, that the decrease of fermentation takes place practically immediately after the addition of the salt, so that the action of cations on enzymes within the cell seems to be excluded, and it becomes probable that we are dealing with a rapid ion exchange at the surface of protoplasm.

(ii) The horizontal levels in the fermentation curves of certain cations are difficult to understand from the hypothesis of an influence on enzymes, especially as these levels lie at different fermentation rates for various cations (see e.g. Ni and Mn in Fig. 7).

(iii) An experiment on *Trockenhefe* with  $\text{Ni}(\text{NO}_3)_2$  showed that fermentation is still 100 % of the blank at  $\log C = 0.90 - 4$ , while it is zero at  $\log C = 0.40 - 3$ . Thus the enzyme systems without the protecting protoplasmic membrane react totally different from the intact cell.

(iv) That the protoplasmic layer protects the cell against cations and that these cations permeate only very slowly is shown by the fact that respiration of the yeast cells is decreased only at high concentration of  $\text{Ni}(\text{NO}_3)_2$  ( $\log C = -2$ ). The discrepancy between the influence of  $\text{Ni}^{++}$  on fermentation and respiration might be explained by the fact that the fermenting cell consumes much more sugar, and that consequently the permeation rate of sugar will be the limiting factor for fermentation.

(v) Some cations (e.g.  $\text{Cu}^{++}$ ) depress fermentation as well as respiration completely in low concentrations. Here it might be supposed that an influence on enzyme systems within the cell is the main cause of this depression, and it would be very dangerous to ascribe this effect to a change in permeability.

So we arrive at the following conclusion. The influence of ions on a metabolic process might be explained in two ways:

(i) In cells which are very permeable to these ions a direct influence on enzyme systems takes place.

(ii) In cells which show a low permeability to ions the influence of these ions on permeability might be the decisive factor.

I feel, in particular, that the appearance of horizontal levels (as in Fig. 7) may be a useful indication to judge which of these two phenomena is the main cause of the influence of the cations on the metabolic process. Practically the same argument was used by Hotchkiss<sup>1</sup> when he rejected the idea that detergents act on enzyme systems within the bacterial cell. When dealing with case (a) one would also expect a time effect, while in the second a marked difference between the influence on aerobic and anaerobic processes must appear (this might also appear in the first case, of course). Transitions from case (ii) to case (i) might in some cases be performed by treating a cell for a long time with a slowly permeating salt. Then eventually the concentration of the cation within the cell will grow high enough to affect certain enzyme systems. Moreover, it seems justified to assume that various cells differ very much as regards their permeability to ions.

I agree with Dr. Lasnitzki that the influence of cations on a metabolic process may only be taken as an indication of changes in permeability if we are sure that we are dealing with the second case. The reverse also holds; one may ascribe the influence of cations on a metabolic process of a living cell to an action on enzyme systems only if one is sure that permeability changes do not enter into the picture.

<sup>1</sup> *Ann. N.Y. Acad. Sci.*, 1944, 46, 479.

**Dr. J. A. Lovern** (*Aberdeen*) said: There are two points I would like to make on Prof. O'Connor's paper. (1) Recent work in Canada by Hoar and Dorchester<sup>1</sup> has shown that heat tolerance in goldfish is dependent on the composition of the body fat and can be markedly altered by feeding the fish on special fat diets. This work seems to lend support to Prof. O'Connor's general thesis.

(2) Prof. O'Connor draws attention to the fall in oxygen consumption in earthworms at 17° C and suggests that this may perhaps be due to the presence of 5 % of lauric acid in the earthworm lipids. The fatty acids of earthworms are a particularly complex mixture and one would have expected the curve for the earthworm to show falls (and perhaps also increases) at numerous points, corresponding to the various acids present in addition to lauric, palmitic and oleic acids.

**Prof. J. M. O'Connor** (*Dublin*) said: I thank Dr. Lovern for directing attention to the work of Hoar and Dorchester. A difficulty is presented by the presence in some animals of unsaturated fatty acids whose surface properties, at least so far as temperature is concerned, are unknown. It is very probable, however, from analogy, that they will have disappeared from the surface at temperatures below that at which oleic acid dissolves and would consequently not produce effects on the oxygen consumption at temperature as high as 30°. In the specific case of the earthworm it has been pointed out that the oxygen consumption begins to fall at about 28° (instead of at 30° in the frog) and it was suggested that this earlier fall might be due to some of the lower unsaturated fatty acids which occur in the worm, but not in the frog.

**Dr. P. R. Rowland** (*London*) said: Would it not be correct to say that about the only interfaces of any importance within the animal cell are between aqueous fluids containing more or less dissolved or dispersed matter on the one hand and proteins or fats on the other? And would a fatty acid behave at either of these interfaces as it does at water-air interfaces, especially as regards temperature effects?

Whether it does so by means of the nervous system or by localized automatic processes such as those postulated in the paper, the body must, of course, control temperature by reference to the physical state of a substance or system of substances. It is possible that the behaviour of fatty acids at water-air interfaces with respect to temperature variation may reflect fundamental properties which are independent of the particular type of phase boundary involved, but I think that this should be established by experiment and not assumed.

**Prof. J. M. O'Connor** (*Dublin*) said: Monolayers of fatty acids have been studied almost exclusively at water-air surfaces. The substitution of another phase for the air might be expected to modify the behaviour of the layer, but not drastically to alter it.<sup>2</sup> It is not to be expected that all speculation about the chemical organization of the cell should be suppressed until its microscopic structure has been fully described and the description universally accepted as correct, and until the behaviour of fatty acid monolayers at all varieties of phase boundaries had been satisfactorily examined. Here speculation has led to experimental tests which, when carried through, show results corresponding to the known behaviour of fatty acid monolayers. This appears to be a legitimate procedure.

<sup>1</sup> Hoar and Dorchester, *Can. J. Res. D*, 1949, **27**, 85.

<sup>2</sup> Alexander, *Ann. Reports*, 1944, **41**, 5.

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**Discussions of the Faraday Society**

No. 7, 1949

# **CHROMATOGRAPHIC ANALYSIS**

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A GENERAL DISCUSSION

ON

# CHROMATOGRAPHIC ANALYSIS

22nd - 24th September, 1949

A GENERAL DISCUSSION on Chromatographic Analysis was held in the Department of Chemistry, Reading University (by kind permission of the Vice-Chancellor), on the 22nd, 23rd and 24th September, 1949. The President, Prof. Sir John Lennard-Jones, K.B.E., F.R.S., was in the Chair and over 250 members and visitors were present.

Among the distinguished overseas members and guests welcomed by the President were the following :—

Dr. J. Boldingh (Zwijndrecht, Holland), Prof. H. Brockmann (Göttingen), Prof. S. Claesson (Uppsala), Prof. T. Dillon (Galway), Miss H. M. Doery (Melbourne), Mr. J. Fahrenfort (Amsterdam), Dr. A. H. Gordon (Stockholm), Dr. Grifols-Lucas (Barcelona), Dr. F. A. Haak (Amsterdam), Dr. J. Th. Hackmann (Amsterdam), Dr. H. Hering (Paris), Miss T. Hofstee (Amsterdam), Dr. Klinkenberg (The Hague), Dr. E. Lederer (Paris), Dr. Stanford Moore (New York), Dr. J. van Ormondt (Delft), Dr. L. Sacconi (Florence), Dr. F. H. Spedding (Iowa), Prof. Arne Tiselius (Uppsala), Dr. A. E. R. Westman (Ontario), Prof. and Mrs. L. Zechmeister (California).



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# CHROMATOGRAPHIC ANALYSIS

## GENERAL INTRODUCTION

BY ARNE TISELIUS

*Received 22nd September, 1949*

It is probably well known to all those present how chromatography, although invented and applied for the first time as early as in 1906 by Tswett, was almost completely neglected for a period of 25 years, and how it was actually rediscovered in 1931 by Kuhn, Winterstein and Lederer, who applied it to the resolution of plant carotene into its components. Then followed a period of rapid development, the importance of which may perhaps best be illustrated by a quotation from Karrer, who in his Congress Lecture to the International Congress of Pure and Applied Chemistry in London in 1947 stated that "... no other discovery has exerted as great an influence and widened the field of investigation of the organic chemist as much as Tswett's chromatographic adsorption analysis. Research in the field of vitamins, hormones, carotinoids and numerous other natural compounds could never have progressed so rapidly and achieved such great results if it had not been for this new method, which has also disclosed the enormous variety of closely related compounds in nature."

As a matter of fact the progress in the field of chromatography during the last 5-10 years has been so striking both with regard to the method itself and to its scope of application that one is tempted to speak about a second discontinuity in the curve which would describe the development of this remarkable method and its importance in all fields of chemistry. The time chosen for this Discussion is therefore particularly suitable and I feel that we all would like to express our thanks to the Faraday Society for organizing a discussion of this matter just now. The number of communications in this field is rapidly increasing and there has hardly been time enough to compare experiences with alternative methods or to discuss the numerous new applications which now seem possible. This meeting should afford an opportunity for this and for other valuable discussions in the field.

Some of the most important recent developments are: the development of partition chromatography, the introduction of efficient ion-exchange materials suitable for chromatographic work, the introduction of improved methods for following the process of separation by continuous physico-chemical measurements or by automatic sample-collectors and the micro-chromatographic methods made possible by using filter paper as a medium for partition chromatography. The combination of chromatographic and tracer methods is only in its beginning but has tremendous possibilities. Although not strictly belonging to chromatographic methods in a narrow sense I would like to add to these examples the counter-current extraction methods developed by Craig.

If one would try to mention some particularly striking examples of newer applications, it is obvious that chromatography is no longer limited to the substances studied by organic chemists and biochemists. The recent successful separations of rare earths and fission products from the uranium piles are particularly good examples of the wide scope of the method. Even

if it is obvious that the separation and purification of organic substances of native or synthetic origin is the main object of most chromatographic work it is now clear that the particular application to the complex mixtures of breakdown products obtained by splitting proteins, polysaccharides, nucleic acids and other large molecules has already given chromatography a prominent place among the tools of the structural chemistry of these extremely important substances. Among the newer applications it should also be noted that some quite promising attempts have been made to apply chromatography to high polymers and to viruses and some other proteins. The adsorption analysis of gases and vapours also seems to have useful applications. Many other examples will be given in the papers presented at this Discussion. No doubt chromatography can now be said to be of essential importance for the whole field of chemistry, and no other separation method has such a wide field of application.

Despite this, one has still the impression that much work in this field is too empirical. That is natural enough, because if a separation is successful there is no immediate need for going deeper into the subject in that particular case. But if the separation is not successful there is every reason to do so. We are still far from the goal of placing chromatographic methods on a rational basis to the same extent as has been done, for example, with fractional distillation. The formal theory of the method has been worked out in detail, and we shall listen to some valuable contributions on this aspect in the first section of this Discussion. In my own laboratory we have found it useful to distinguish between three main types of chromatography, namely, frontal analysis, elution analysis and displacement analysis, as the conditions for the successful operation of a column are quite different in these three cases. The theoretical treatment of elution in partition chromatography, as worked out by Martin and Synge, is particularly illuminating since it describes the action of a partition column as a counter-current extraction arrangement with a very large number of theoretical plates. The fundamental characteristics of a column are of course the affinity between the solute and the particles of the column in the medium used for the separation, that is, *the adsorption or the partition isotherm*, or, with a common denominator, the "distribution" isotherm, and that naturally will enter into any theoretical treatment of the operations as the chief unknown variable. One of the great advantages of partition chromatography is that this function can be predicted reasonably well on the basis of known solubilities in the two phases. The situation is not quite as favourable in adsorption chromatography. It appears to be very difficult to prepare reproducible adsorbents if they are to be sufficiently powerful to be used in chromatographic columns. The influence of small impurities on such large active surfaces is naturally very great. In my opinion, however, one should not be too pessimistic about the possibilities here, because one has the impression that on the whole the *relative* magnitudes of adsorption affinity in a series of substances will be fairly constant, even if the capacity may vary from batch to batch. That is certainly true for a number of active charcoals, as shown, for example, by Claesson in his work on fatty acids where Traube's rule determines the variation of adsorption with molecular size in an homologous series. Similar regularities have been found with amino acids and sugars on charcoal. The change of affinity by changing the composition of the solvent is, of course, of the greatest importance in the "development" of chromatograms, and here the change from non-polar to polar solvents (or the reverse) has been a standard practice in chromatography for many years. Such effects can be predicted qualitatively, at least with a reasonable degree of certainty, and have been studied in detail, for example, by Trappe in his work on the "elu-

tropic series" in the elution of lipids. The ion-exchange resins, as, for example, Amberlite, Wofatite and Dowex, are really to a certain extent "adsorbents made to order" and have already proved to be extremely useful both for separation of inorganic ions, of amino acids and many other substances. On the other hand, these adsorbents show a specificity which is not only dependent upon electrochemical properties. It would be highly desirable to study such phenomena in detail, and to investigate the influence of the size of pores in these materials and the possibility of preparing very porous resins for use with larger molecules.

Speaking of adsorbents which can be made to have a certain specificity, F. H. Dickey has recently published a paper<sup>1</sup> in which silica gel was precipitated together with, for example, methyl orange. The resulting adsorbent, after removing the indicator, had a very marked specific affinity for this substance. If this principle would prove to be of general applicability, it would, of course, mean a great step forward.

Mixed solvents, obtained, for example, by the addition of a polar solvent to a non-polar, working with a polar adsorbent, are generally used for elution or for the development of chromatograms, as has already been mentioned. This is probably due to a displacement effect, as was already realized by Tswett in his first communication. The reverse effect also must exist, although it has not been studied in great detail. Thus addition of a second component to the medium must, under certain circumstances, promote the adsorption of the solute. This component may be adsorbed in a polymolecular layer, which may have an affinity for the solute, which the original adsorbent did not have. We are here on the borderline between adsorption and partition chromatography—in the latter case the layer has extended so much that one can speak of a separate phase. In discussing the various forms of partition chromatography and the eventual influence of adsorption in these, I believe it is well to keep in mind that such phenomena are likely to occur. There is also the possibility that the substance added to the medium will not appreciably change the properties of the adsorbent but will change the thermodynamic potential of the *solute* by change in electrolytic dissociation, by complex formation or by other effects which express themselves as a change in solubility of the solute. This is made use of in certain cases of elution by modification in pH, by addition of citrate or other complex-forming agents. But here also the reverse effect has been observed. Thus any agent which will decrease the solubility of a substance should increase its adsorption, provided that the adsorbent is not influenced. Some dye-stuffs which are normally not adsorbed on filter paper will do so on addition of salting-out agents, for example, ammonium sulphate, and good chromatograms can be obtained by elution with water. Some proteins show this phenomenon too, especially on Celite and silica gel, and it has been used for the chromatographic separation of some viruses.

We thus have in our hands varying methods of modifying the adsorption affinity and specificity of adsorbents by addition of suitable substances. With the enormous number of alternative combinations possible it is natural that this field has as yet been very little explored, but it is well to remember that it is no longer necessary to feel oneself limited to the adsorption characteristics of the commercially available adsorbents as they are obtained from the manufacturer. However, for the application in chromatography it is essential not only to have adsorbents of satisfactory affinity and specificity, they must also have a reasonably high capacity and be capable of acting reversibly at a fairly fast rate. That often limits the choice quite considerably. For the elution procedure it is very essential to avoid the

<sup>1</sup> *Proc. Nat. Acad. Sci. (Washington)*, 1949, **35**, 227.

unpleasant lagging behind and broadening of the zones which is usually called "tailing." The tailing may be due to a too slow establishment of equilibrium, but just as often it is the consequence of the adsorption or partition isotherm being curved which makes the higher concentrations of a zone travel faster than the lower. A great advantage of partition chromatography is the fact that the isotherm is generally almost linear. With adsorption this is not so often the case, at least not for strongly adsorbed substances. At low concentrations, however, many adsorption isotherms are linear, as required by the Langmuir theory. This is, of course, ultimately a question of the homogeneity of the surface with respect to affinities, but also a question of the space available and when saturation is approached the isotherm is bound to bend and become concave, both in adsorption and partition processes. In such cases it is evident that elution cannot separate two components A and B (A more strongly bound than B) if the lower concentrations of B would show a higher relative adsorption or partition than the higher concentrations of A. A great advantage of displacement and frontal analysis is that the stationary concentration of the zones is constant even in such a case and thus a separation can be realized, and very large columns may be used without any spreading out of the zones.

In the development of chromatographic technique during recent years there has been a tendency to use the so-called "liquid" chromatogram method for following the separation. This method has great advantages as it makes one independent of optical methods for observing the zones directly on the column. The new automatic sample-collectors of Moore and Stein have proved extremely useful for this purpose. The other alternative of continuous observation of some convenient physicochemical property related to the concentration of the percolate as it leaves the column has been studied particularly in my own laboratory and also in a number of other places. These methods are particularly useful in the study of displacement and frontal analysis when the number of observations must be very large to get the concentration-volume curve accurately reproduced. Some combination of both methods would seem to be the most ideal procedure, but it is highly desirable to increase the sensitivity of the optical (or other) methods applied to be able to deal with the very low concentrations used in most work in partition chromatography. This is also essential for the proper interpretation of frontal analysis curves, where one wants to use low concentrations in order to avoid displacement effects as far as possible.

The direct observation of zones on the column has many advantages, and several interesting suggestions for new procedures have been made for the observation of colourless substances. Ultra-violet absorption observation of columns in quartz tubes offers many interesting possibilities, but one is, of course, then always limited in the choice of the adsorbent. The filter-paper chromatography offers some problems of this kind—there it now seems particularly important to develop the method further in the quantitative direction.

Chromatography has so far been based only upon adsorption and partition phenomena, but it is obvious that any phenomenon which would—in a specific manner—influence the rate at which a zone of a substance travels through a column might be utilized for separation purposes. Electrophoresis and ionophoresis are related processes and have been utilized in a way analogous to chromatographic separation, using the column chiefly as a stabilizing medium to avoid gravitational or thermal disturbances. A combination of chromatography and electrophoresis has been tried by Strain. It seems as if electrophoretic separation in columns of ion-exchange resins would offer interesting possibilities, as it must be expected that the specific affinity

of the substances to the resin will strongly influence their migration. There is another possibility, which I have several times discussed with Dr. Martin and Dr. Synge, namely, to make use of differences in the frictional resistance in a gel. Such separations would mainly depend upon differences in molecular size, and would thus be very valuable in many cases, but offers great technical difficulties. Some of the separations observed in ionophoresis in gels may be due to such effects, in part at least.

I am afraid that in this Introduction I have dealt more with methods than with applications, but it is perhaps not necessary to this audience to exemplify further the possibilities of a method of such an enormously wide scope as chromatography. The various titles of the many papers to be presented at this Discussion and the presence here of representatives of all fields of chemistry and from many countries is a proof as good as any of this point. I shall be very happy if the remarks I have made here would stimulate the Discussion, to which we are all looking forward with the greatest interest.

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## I. PHYSICOCHEMICAL PRINCIPLES AND THEIR UTILIZATION

### INTRODUCTORY PAPER

BY STIG CLAESSION

*Received 2nd September, 1949*

The importance of chromatography is still rapidly increasing as can be clearly seen from the great number of very interesting contributions to this Discussion. Papers have been presented to this Section which have deepened our understanding of the chromatographic method and important advances have also been made in the experimental application of these ideas.

It is certainly true that well-designed experimental arrangements are of the utmost importance when difficult separations are attempted. Remarkable results can be achieved in this way which is evident from many contributions to this Discussion. A typical example of this kind of work is also Moore and Stein's experiments<sup>1</sup> on the separation of amino acids, some of the most carefully planned and beautiful experiments in chromatography ever published.

However, if a problem can be solved in different ways, the simplest way is always the best. A simple fraction collector is therefore often quite as useful as a more intricate apparatus for the continuous recording of the concentration of the effluent, and in many cases the inspection of the column with an ultra-violet lamp is quite satisfactory, particularly when the quenching of the fluorescence of the adsorbent is observed.<sup>2 3</sup>

The most important factor in all chromatographic work is, of course, the properties of the adsorbent in the column. Nothing can therefore ever

<sup>1</sup> Moore and Stein, *J. Biol. Chem.*, 1948, **176**, 337, 367; 1949, **178**, 53, 79

<sup>2</sup> Sease, *J. Amer. Chem. Soc.*, 1947, **69**, 2242.

<sup>3</sup> Brockmann and Volpus, *Ber.*, 1947, **80**, 77.



compete in importance with the introduction of new and powerful adsorbent columns and it is also well known to everybody that the greatest progress in chromatography in recent years is due to the introduction of two new types of adsorbent, the partition column and the ion-exchange column.

It is therefore always of the greatest interest to follow the introduction of new principles for controlling the adsorption process either by changing the adsorbents or solvents used. Several very interesting papers dealing with such problems are found here.

In this connection a paper published by Dickey should be mentioned.<sup>4</sup> Following some ideas of Pauling he was able to show that silica gel precipitated in the presence of, e.g., propyl orange after washing had a greater adsorption affinity for that compound than for methyl, ethyl or butyl orange. If such results can be improved and extended they will be of such value that their importance can hardly be overestimated.

There are, however, two factors about which so little is known that they almost prevent progress in certain branches of chromatography. One is the problem of the connection between adsorption and chemical structure. Some progress has certainly been made in this field, but much still remains to be done before we can choose the adsorbents in a scientific way instead of using our intuition which is almost the best we can do to-day.

The other factor which prevents the progress of chromatography is the lack of reproducible adsorbents of constant quality standardized in suitable ways. This difficulty could certainly be overcome by the chemical industry to-day if the importance of this factor was made sufficiently clear to manufacturers of chemical products by a suitable group of scientists, e.g., those present at this Discussion.

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<sup>4</sup> Dickey, *Proc. Nat. Acad. Sci.*, 1949, **35**, 227.

## THEORY OF CHROMATOGRAPHY

### VII. The General Theory of Two Solute following Non-linear Isotherms

BY E. GLUECKAUF

*Received 2nd June, 1949*

The movement of solutes in an ideal adsorbing column during a chromatographic separation depends on the adsorption isotherm, i.e., the amounts  $f_1$  and  $f_2$  adsorbed in equilibrium with the concentrations  $c_1$  and  $c_2$  of the two solutes.

Calculation leads to the fundamental relationship,  $df_1/dc_1 = df_2/dc_2$ . This results from the conservation of solute mass in an adsorbing column under the normal chromatographic conditions, and it requires that coexistent  $c_1$  and  $c_2$  are functions of each other only. The solution of the equation leads to a family of curves (characteristics) in a system with the co-ordinates  $c_1$  and  $c_2$ . Co-existing concentrations of the two solutes occurring in the same chromatographic boundary lie on one such curve and this curve is uniquely determined by the composition of the original solution and by the solution used for developing the chromatogram. The sequence of concentrations in the column (i.e., the path followed in the diagram of characteristics) as well as any new concentration plateaux developing spontaneously, and the occurrence of sharp or diffuse boundaries, can be predicted by the application of a few simple rules, which are deduced. In this way a unified method is provided which can predict the chromatographic development of any binary mixture of which the binary adsorption isotherms are known. This applies both to solvent development and displacement development.

**General Theory.**—If a small amount of solvent  $dv$  containing an adsorbable solute of concentration  $c$  passes through a narrow section of the column weighing  $dx$  g., then conservation of mass requires that the amount of solute lost from the solvent during passage of this section must have increased the solute content of this section ( $f(c)dx$ ), see Fig. 1.

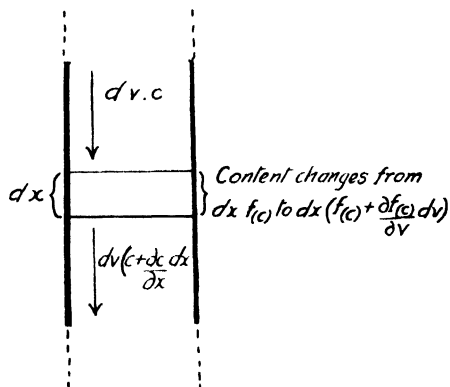


FIG. 1.—Balance of solute mass for a solution flowing through a column of sorbent.

This, as has been shown by Wilson,<sup>1</sup> must be true for every individual solute, and leads to the differential equation

$$\left(\frac{\partial c_i}{\partial x}\right)_v + \left(\frac{\partial f_i}{\partial v}\right)_x = 0. \quad (1)$$

In the further development of this function it must be taken into consideration that  $f_i$  is usually a function of the concentrations of all the solutes present in the solution:

$$f_i = f_i(c_1, c_2, \dots, c_i). \quad (2)$$

For a sharp boundary, the conservation of mass requires that

$$dv \cdot \Delta c_i = dx \cdot \Delta f_i, \quad (3)$$

and as this applies to all the solutes present, the movement of such a sharp boundary can be expressed in terms of any of the solutes:

$$\frac{dx}{dv} = \left(\frac{\Delta c_1}{\Delta f_1}\right)_x = \left(\frac{\Delta c_2}{\Delta f_2}\right)_x = \text{etc.} \quad (4)$$

For a diffuse boundary, eqn. (1) can be transformed as was done by de Vault<sup>2</sup>:

$$\left(\frac{dv}{dx}\right)_{c_1} = \left(\frac{df_1}{dc_1}\right)_{x,v} \quad (5a)$$

and

$$\left(\frac{dv}{dx}\right)_{c_2} = \left(\frac{df_2}{dc_2}\right)_{x,v} \quad \text{etc.} \quad (5b)$$

For further discussion we confine ourselves to only two solutes and we assume that the isotherms  $f_1$  and  $f_2$  are such that

$$(I) \quad \begin{aligned} &\text{for } c_1 = 0, f_1 = 0, \\ &\text{for } c_2 = 0, f_2 = 0 \end{aligned}$$

and

$$(II) \quad \text{that } \partial f_2 / \partial c_1 \text{ has the same sign as } \partial f_1 / \partial c_2.$$

The first assumption is obvious and the Gibbs-Duhem equation requires that the last assumption is always satisfied.

<sup>1</sup> Wilson, *J. Amer. Chem. Soc.*, 1940, **62**, 1583.

<sup>2</sup> De Vault, *ibid.*, 1943, **65**, 532.

Two situations are possible :

(i) either there is a functional relationship between  $c_1$  and  $c_2$  alone, not involving  $v$  and  $x$ . Then we have a relationship of the type,

$$c_1 = \varphi(c_2) \quad . \quad . \quad . \quad . \quad . \quad (6)$$

or (ii) there is no such relationship.

A mathematical analysis of the properties of the system (5a, b) shows that case (i) holds only for the very simplest boundary conditions, i.e., if a solution of constant concentration is fed into an adsorption column which itself contains the solutes in another constant concentration. Fortunately this is the rule in normal chromatographic procedure. One starts with the boundary conditions  $c_1 = 0$ ,  $c_2 = 0$  (empty column), then adds the solution to be separated into its constituents with the constant composition ( $c_1 = c_1^\circ$ ,  $c_2 = c_2^\circ$ ), and then develops this band of constant concentrations with another solution where again  $c_1 = 0$  and  $c_2 = 0$ . As soon as we introduce special boundary conditions, e.g., an initial distribution of solutes in the column of a type which does not arise from feeding a solution of constant composition, we have case (ii). In these cases it will not be possible to find a simple dependence between the variations of  $x$  and  $v$  due to the fact that there is a varying  $c_1 - c_2$  combination which does not permit the function  $df/dc_i$  to be expressed in terms not containing  $x$  and  $v$  themselves. For all practical purposes case (ii) will only arise when, in the rare case of isotherms convex against the  $c$ -axis (when a diffuse front is produced), the band is developed with a different solvent. It is not believed that these cases are sufficiently important to warrant an exceedingly involved discussion.

In the event that there is a functional relationship (6) between  $c_1$  and  $c_2$  (case (i)), eqn. (5a, b) become

$$\left(\frac{dv}{dx}\right)_{c_1, c_2} = \left(\frac{df_1}{dc_1}\right) \quad . \quad . \quad . \quad . \quad (7)$$

$$\left(\frac{df_1}{dc_1}\right) = \left(\frac{df_2}{dc_2}\right) \quad . \quad . \quad . \quad . \quad (8)$$

and eqn. (8) makes it possible to derive this relationship between coexistent values of  $c_1$  and  $c_2$  (eqn. 6).

Partial differentiation of both sides of (8) leads to

$$\left(\frac{dc_1}{dc_2}\right)^2 + \frac{\frac{\partial f_2}{\partial c_2} - \frac{\partial f_1}{\partial c_1}}{\frac{\partial f_2}{\partial c_1}} \cdot \left(\frac{dc_1}{dc_2}\right) - \left(\frac{\frac{\partial f_1}{\partial c_2}}{\frac{\partial f_2}{\partial c_1}}\right) = 0, \quad . \quad . \quad (9)$$

which form was first used by Offord and Weiss.<sup>3</sup> For any given  $c_1$  and  $c_2$ , the quadratic eqn. (9) leads to two alternative values for  $dc_1/dc_2$ . In view of assumption II with respect to  $f_1(c_1, c_2)$  and  $f_2(c_1, c_2)$ , the free term in this equation is negative. Consequently the two roots of the quadratic are both real, one being positive, the other negative.

Eqn. (9) makes it possible to construct the curves of  $c_1$  as function of  $c_2$  for any given isotherm and for any given starting point  $c_1^\circ, c_2^\circ$ , the next points on the curve being given by

$$\left. \begin{aligned} c_2' &= c_2^\circ - \Delta c_2 \quad \text{and} \quad c_1' = c_1^\circ - \left[ \frac{dc_1}{dc_2} \right]_{c_1^\circ, c_2^\circ} \cdot \Delta c_2, \\ c_2'' &= c_2' - \Delta c_2 \quad \text{and} \quad c_1'' = c_1' - \left[ \frac{dc_1}{dc_2} \right]_{c_1', c_2'} \cdot \Delta c_2, \end{aligned} \right\}$$

<sup>3</sup> Offord and Weiss, *Nature*, 1945, **155**, 725.

and so on. In this manner we can determine the two curves passing through the point  $c_1^0, c_2^0$ , with positive slope, by using first the positive values of  $dc_1/dc_2$ , and with negative slopes, by using the negative values. Starting with all possible points  $c_1^0, c_2^0$  in the quadrant  $c_1 > 0, c_2 > 0$ , we shall obtain two whole families of curves, covering the entire quadrant. These families of curves are known as the "families of characteristics" of the differential eqn. (8) (Fig. 2). Only such combinations of  $c_1$  and  $c_2$  can occur in the same chromatographic boundary which lie on the same characteristic.

(Instead of constructing the  $c_1$ - $c_2$  characteristics, it is sometimes more convenient to use the  $f_1$ - $f_2$  characteristics. The choice depends on the equation for the adsorption isotherm. All the general conclusions drawn for the  $c$  characteristics apply equally to the  $f$  characteristics, though the equations are obviously modified.)

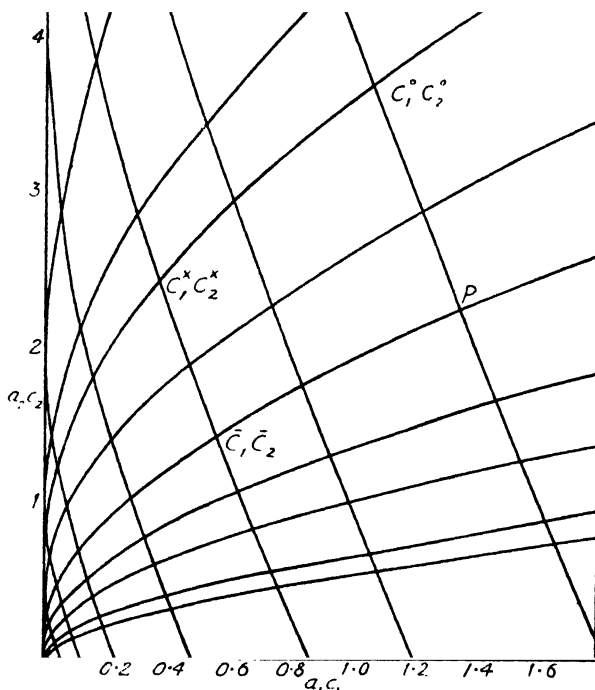


FIG. 2.— $c_1$ - $c_2$  diagram of characteristics for the adsorption isotherms

$$\begin{aligned} a_1 c_1 &= f_1 (f_1 + f_2) \quad \text{for} \quad a_2 = 2. \\ a_2 c_2 &= f_2 (f_1 + f_2) \end{aligned}$$

Only sets of concentrations lying on the same characteristic can coexist in the same chromatographic boundary.

The fact that the entire quadrant  $c_1 > 0, c_2 > 0$  is covered by these curves is of importance, because it implies that the "envelope" of these curves, which itself represents a solution to the differential equation, lies outside this quadrant and therefore does not include cases where both concentrations have values above zero, which alone are of practical significance. Thus no ambiguity is possible.

It is also important to note that a particular characteristic is defined by only one concentration set  $c_1, c_2$ , so that, if a column is filled with a solution  $c_1^0, c_2^0$  and then developed with a solution  $\bar{c}_1, \bar{c}_2$ , we cannot as a rule

expect that these two solutions lie on the same characteristic. Each solution will possess its own pair of characteristics, which is of some importance for the form of chromatographic bands of two solutes (Fig. 3).

Thus, when a solution is poured on a column which has previously been treated with another solution containing the same solutes at different concentrations, they will, as a rule, produce two separate boundaries, separated by a band of constant concentrations  $c_1^* c_2^*$ , which correspond to the point of intersection of two characteristics passing through the points  $c_1^0 c_2^0$  and  $\bar{c}_1 \bar{c}_2$ , respectively.

But before discussing this in detail, it is necessary to return once more to eqn. (7) which concerns the  $v$ - $x$  relationship, i.e., the movement of a point of given concentrations  $c_1 c_2$ .

We can write

$$\left(\frac{dv}{dx}\right)_{c_1 c_2} = \frac{df_1}{dc_1} = \frac{\partial f_1}{\partial c_1} + \frac{\partial f_1}{\partial c_2} \cdot \left(\frac{dc_2}{dc_1}\right) \quad (10)$$

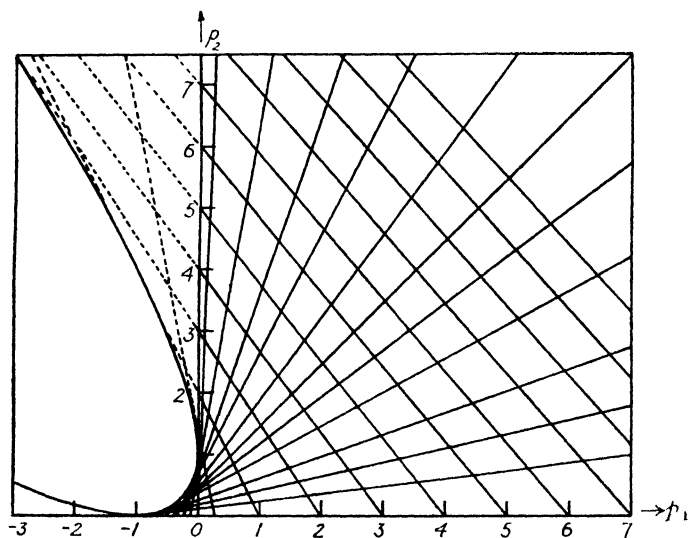


FIG. 3.— $p_1$ - $p_2$  diagram of characteristics for any Langmuir isotherm showing the parabolic envelope of the linear characteristics.

If we take the case of a binary isotherm where  $\partial f_1 / \partial c_2$  is negative (which is almost invariably the case), then it follows that  $dv/dx$  for a given point  $c_1 c_2$  is always smaller, and consequently the rate of movement  $dx/dv$  of a point  $c_1 c_2$  is always larger for positive values of  $dc_2/dc_1$  than for negative ones.

A concentration plateau  $c_1^* c_2^*$  in a chromatogram is only stable and growing when its forward point moves faster than its rear. It follows from this that a concentration plateau  $c_1^* c_2^*$  is produced spontaneously in a boundary, if the boundary section in front of it travels along the positive characteristic of  $c_1^* c_2^*$  (i.e., towards  $c_1^0 c_2^0$  in Fig. 2) while the rear travels along a characteristic where  $dc_1/dc_2$  is negative (or zero) (RULE 1).

This eliminates any ambiguity as regards the route which a boundary can take between two points, as the alternative concentration plateau at the point P (Fig. 2) cannot form spontaneously, if the solution  $\bar{c}_1 \bar{c}_2$  follows the solution  $c_1^0 c_2^0$ . (If, however,  $\bar{c}_1 \bar{c}_2$  is in front of  $c_1^0 c_2^0$ , then for the same reason the boundary contains a concentration plateau near P.)

When changes occur in a column along the characteristics of the equation

$$\frac{df_1(c_1, c_2)}{dc_1} = \frac{df_2(c_1, c_2)}{dc_2}, \quad (8)$$

then  $c_2$  is always a function of  $c_1$  defined by the original solution ( $c_1^\circ, c_2^\circ$ ) of the particular case. We can therefore write

$$\frac{dv}{dx} = \frac{df_1(c_1, c_2)}{dc_1} = \frac{dF_1(c_1)}{dc_1}, \quad (11)$$

where  $F_1$  is a new function of  $c_1$  only, which fully corresponds to the adsorption isotherm  $f(c)$  in the case of a single solute, with the only exception that the function  $F_1$  depends also on the initial conditions  $c_1^\circ, c_2^\circ$ . Counting  $v$  from the change-over to the new solution  $\bar{c}_1, \bar{c}_2$  we can integrate to

$$x = v \int \frac{dF_1(c_1)}{dc_1} = \frac{v}{F_1'} = v \int \frac{df_1(c_1, c_2)}{dc_1}, \quad (12a)$$

and

$$x = v \int \frac{dF_2(c_2)}{dc_2} = \frac{v}{F_2'} = v \int \frac{df_2(c_1, c_2)}{dc_2}. \quad (12b)$$

Once the transformation to the single solute equation has been made, the sequence of chromatographic band movements follows in a straightforward way, as has been described in previous publications.<sup>4, 5, 6</sup>

The question where a diffuse or a sharp boundary arises is again very similar to the case of a single solute as neither  $dc_1/dx$  nor  $dc_2/dx$  must become positive in a frontal boundary or negative in a rear boundary. It thus depends on  $F_1''$  and on  $F_2'' = \left[ \frac{d^2 F_2}{dc_2^2} \right]_v$  which type of boundary occurs.

But, unlike the case of a single solute, it requires some knowledge of the characteristics to see under what conditions  $F_1''$  and  $F_2''$  are positive or negative.

**Langmuir Isotherms.** The general case can be best understood by illustrating it with the Langmuir isotherm, which offers the advantage that eqn. (8) can be directly solved by integration, so that general expressions can be obtained for all functions, and for the movements of all points in the chromatographic band.

Writing the Langmuir isotherm in the form

$$f_1 = \frac{a_1 c_1}{1 + \beta a_1 c_1 + \beta a_2 c_2}, \quad (13a)$$

and

$$f_2 = \frac{a_2 c_2}{1 + \beta a_1 c_1 + \beta a_2 c_2}, \quad (13b)$$

where  $a_2 > a_1$  and  $\beta$  are all some positive constants. It is convenient to introduce

$$\beta a_1 = b_1 \text{ and } \beta a_2 = b_2 \text{ and } a_2/a_1 = b_2/b_1 = K.$$

(As shown by Kemball, Rideal and Guggenheim<sup>7</sup> the use of different values of  $\beta_1$  and  $\beta_2$  offends the Gibbs-Duhem law.)

For these isotherms (8) reduces to

$$b_1(1 + b_2 c_2)dc_1 dc_2 + b_1 b_2 c_2 (dc_1)^2 - b_1 b_2 c_1 (dc_2)^2 - b_2(1 + b_1 c_1)dc_1 dc_2. \quad (14)$$

<sup>4</sup> Glueckauf, *Proc. Roy. Soc. A*, 1946, **186**, 35.

<sup>5</sup> Glueckauf, *J. Chem. Soc.*, 1947, 1321.

<sup>6</sup> Coates and Glueckauf, *J. Chem. Soc.*, 1947, 1309.

<sup>7</sup> Kemball, Rideal, and Guggenheim, *Trans. Faraday Soc.*, 1948, **44**, 952.

Putting for brevity

$$\frac{b_1 b_2}{b_2 - b_1} \cdot c_1 = p_1, \quad . \quad . \quad . \quad (15a)$$

and

$$\frac{b_1 b_2}{b_2 - b_1} \cdot c_2 = p_2, \quad . \quad . \quad . \quad (15b)$$

and dividing by  $(dp_2)^2$ , we obtain

$$p_2 \left( \frac{dp_1}{dp_2} \right)^2 - \left( 1 + p_1 - p_2 \right) \cdot \frac{dp_1}{dp_2} - p_1 = 0. \quad . \quad . \quad (16)$$

Differentiating again with respect to  $p_2$

$$\left[ 2 p_2 \cdot \frac{dp_1}{dp_2} - \left( 1 + p_1 - p_2 \right) \right] \cdot \frac{d^2 p_1}{dp_2^2} = 0. \quad . \quad . \quad (17)$$

Hence, either  $p_1$  is a linear function of  $p_2$  or

$$2 p_2 \cdot \frac{dp_1}{dp_2} = 1 + p_1 - p_2 \quad . \quad . \quad . \quad (18)$$

Eliminating  $dp_1/dp_2$  in (16) by means of (18), we obtain

$$(1 + p_1 - p_2)^2 + 4 p_1 p_2 = 0. \quad . \quad . \quad (19)$$

This function is a parabola which merely touches the quadrant  $c_1 > 0$ ,  $c_2 > 0$  at the point  $c_1 = 0$ ,  $c_2 = \frac{b_2 - b_1}{b_1 b_2}$ , lying otherwise entirely outside this quadrant. It does not, therefore, apply to any real concentrations and need not concern us.

The only solutions of (16) where  $p_1$  and  $p_2$  are both positive arise therefore from

$$\frac{d^2 p_1}{dp_2^2} = 0, \quad . \quad . \quad . \quad (20)$$

where  $p_1$  is a linear function of  $p_2$ . This leads to

$$p_1 = \lambda \cdot p_2 - \lambda / (1 + \lambda), \quad . \quad . \quad . \quad (21a)$$

or, returning to the concentrations,

$$c_1 = \lambda c_2 - \frac{(b_2 - b_1)}{b_1 b_2} \cdot \frac{\lambda}{(1 + \lambda)}. \quad . \quad . \quad (21b)$$

Here  $\lambda$  is an integration constant, depending on the given starting concentrations. In a system of  $c_1$  and  $c_2$  co-ordinates, eqn. (21a or b) represents straight lines which envelop the parabola of eqn. (19), and this makes it easy to construct the lines (21a or b) geometrically (Fig. 3). If we are given some point  $c_1 c_2$  we can draw through this point the two tangents to the curve (19) and these tangents give us the two characteristics passing through the point  $c_1 c_2$ , which contain all the coexistent concentration sets. From the fact that the lines (21) are tangents to a convex curve, it follows that no two different lines possess the same value  $\lambda$ . Consequently  $\lambda$ , which is the slope of the lines (21a), can serve as a parameter to distinguish one characteristic from another.

As two tangents pass through every point  $c_1 > 0$ ,  $c_2 > 0$ , we can introduce the two  $\lambda$  values as parameters characterizing every mixture  $c_1 c_2$ . We take the positive  $\lambda$ s as the parameter  $\mu$ , and the negative ones as the parameter  $\nu$ . The positive-slope family of characteristics has values of  $\mu \geq 0$  while the negative-slope family has values of  $\nu$  between 0 and  $-1$ .

If we are given a point  $c_1 c_2$ , then  $\mu$  and  $\nu$  can be determined by solving (21b), for  $\lambda$  in

$$\lambda^2 + \lambda \left( 1 - \frac{c_1}{c_2} - \frac{(b_2 - b_1)}{b_1 b_2 c_2} \right) - \frac{c_1}{c_2} = 0, \quad . \quad . \quad . \quad (21c)$$

and identifying the positive root of the quadratic with  $\mu$  and its negative root with  $\nu$ .

The values of  $\mu$  as function of  $c_1/c_2$  and  $(b_2 - b_1)/b_1 b_2 c_2$  are shown in Fig. 2.<sup>4</sup>

From the equations of the two characteristics (see (21b)),

$$p_1 = \mu p_2 - \mu/(1 + \mu),$$

and

$$p_1 = \nu p_2 - \nu/(1 + \nu),$$

we can solve for  $p_1$  and  $p_2$ , or  $c_1$  and  $c_2$ .

$$c_1 = \frac{b_2 - b_1}{b_1 b_2} \cdot \frac{-\mu \cdot \nu}{(1 + \mu)(1 + \nu)}, \quad . \quad . \quad . \quad (22)$$

$$c_2 = \frac{b_2 - b_1}{b_1 b_2} \cdot \frac{1}{(1 + \mu)(1 + \nu)}, \quad . \quad . \quad . \quad (23)$$

which gives us the values of  $c_1$  and  $c_2$ , in terms of the parameters  $\mu$  and  $\nu$ . Both expressions are positive, as  $\nu$  is negative and  $> (-1)$ .

We can also express  $f_1(c_1 c_2)$  and  $f_2(c_1 c_2)$  as functions of the parameters  $\mu$  and  $\nu$ :

$$f_1 = -\mu \cdot \nu \frac{b_2 - b_1}{\beta b_1 (\mu + K)(\nu + K)}, \quad . \quad . \quad . \quad (24)$$

$$f_2 = K \frac{b_2 - b_1}{\beta b_1 (\mu + K)(\nu + K)}, \quad . \quad . \quad . \quad (25)$$

we then obtain the movement of a point of concentration  $c_1 c_2$ :

$$\left[ \frac{\Delta x}{\Delta v} \right]_{c_1 c_2} = \frac{dc_2}{df_2} = \left[ \frac{\partial c_2}{\partial \nu} \right]_{\mu} / \left[ \frac{\partial f_2}{\partial \nu} \right]_{\mu} \quad . \quad . \quad . \quad (26)$$

along the positive characteristic,

$$\text{and} \quad = \left[ \frac{\partial c_2}{\partial \mu} \right]_{\nu} / \left[ \frac{\partial f_2}{\partial \mu} \right]_{\nu} \quad . \quad . \quad . \quad (27)$$

along the negative characteristic, by partially differentiating (23) and (25) with respect to  $\nu$  or  $\mu$ . Thus we obtain the simple functions:

$$\left[ \frac{\Delta x}{\Delta v} \right]_{c_1 c_2 \mu} = \frac{1}{a_2 K} \left( \frac{\mu + K}{\mu + 1} \right) \left( \frac{\nu + K}{\nu + 1} \right)^2 \quad . \quad . \quad . \quad (28)$$

(See Fig. 4 (a),  $A \rightarrow B$  or  $D \rightarrow C$ .)

$$\left[ \frac{\Delta x}{\Delta v} \right]_{c_1 c_2 \nu} = \frac{1}{a_2 K} \left( \frac{\mu + K}{\mu + 1} \right)^2 \left( \frac{\nu + K}{\nu + 1} \right) \quad . \quad . \quad . \quad (29)$$

(See Fig. 4 (a),  $D \rightarrow A$  or  $C \rightarrow B$ .)

It is easy to see from this that the movement along the positive characteristic proceeds faster than along the negative one, as was already predicted generally for isotherms where  $\partial f_1 / \partial c_2$  and  $\partial f_2 / \partial c_1$  are negative.

The condition that we have a sharp boundary requires that  $dc/dx$ , deduced from (28) or (29), is positive at a falling concentration (e.g., when forming



the front of a band) and negative at a rising concentration (e.g., as rear of a band).

Using 
$$\left[ \frac{dc}{dx} \right]_{\mu, v} = \left[ \frac{\partial c}{\partial v} \right]_{\mu} \left/ \left[ \frac{\partial x}{\partial v} \right]_{\mu, v} \right.,$$

and 
$$\left[ \frac{dc}{dx} \right]_{\nu, v} = \left[ \frac{\partial c}{\partial \mu} \right]_{\nu} \left/ \left[ \frac{\partial x}{\partial \mu} \right]_{\nu, v} \right.,$$

as all variations must occur along one of the characteristics, we obtain by differentiating (22), (23), (28) and (29) for constant  $v$  :

$$\left[ \frac{dc_1}{dx} \right]_{\mu, v} = \frac{\mu \cdot (v + 1)}{D} = \text{always positive}, \quad . \quad . \quad (30)$$

where  $D = 2\beta (\mu + K)(v + K) \cdot v/K$  ;

$$\left[ \frac{dc_1}{dx} \right]_{\nu, v} = \frac{v \cdot (\mu + 1)}{D} = \text{always negative} \quad . \quad . \quad (31)$$

$$\left[ \frac{dc_2}{dx} \right]_{\mu, v} = \frac{(v + 1)}{D} = \text{always positive} \quad . \quad . \quad (32)$$

$$\left[ \frac{dc_2}{dx} \right]_{\nu, v} = \frac{\mu + 1}{D} = \text{always positive}. \quad . \quad . \quad (33)$$

For a single solute isotherm of the Langmuir type  $[dc/dx]_v$  is always positive ; we can, therefore, conclude from the above information (30-33) that the more strongly adsorbed solute II behaves in the presence of solute I, as far as type of boundary is concerned, in the same way as if solute I were not there at all. This means that *where the more strongly adsorbed solute  $c_2$  increases along the column we have a diffuse boundary, while where it changes to a lower concentration we have a sharp boundary* (RULE 2). Fig. 4 illustrates the practical aspect of this rule for the properties of various boundaries.

The rate of movement of these sharp boundaries (s.b.) is given by

$$\left[ \frac{\Delta x}{\Delta v} \right]^{\text{s.b.}} = \frac{\Delta c_1}{\Delta f_1} = \frac{\Delta c_2}{\Delta f_2},$$

and replacing the  $c$  and  $f$  values by  $\mu$  and  $v$  from eqn. (32-35) leads to

$$\left[ \frac{\Delta x}{\Delta v} \right]_{\mu}^{\text{s.b.}} = \frac{1}{a_2 K} \left( \frac{\mu + K}{\mu + 1} \right) \left( \frac{v_1 + K}{v_1 + 1} \right) \left( \frac{v_2 + K}{v_2 + 1} \right) \quad . \quad . \quad (34)$$

(See Fig. 4 (a), B  $\rightarrow$  A or C  $\rightarrow$  D.)

$$\left[ \frac{\Delta x}{\Delta v} \right]_{\nu}^{\text{s.b.}} = \frac{1}{a_2 K} \left( \frac{\mu_1 + K}{\mu_1 + 1} \right) \left( \frac{\mu_2 + K}{\mu_2 + 1} \right) \left( \frac{v + K}{v + 1} \right) \quad . \quad . \quad (35)$$

(See Fig. 4 (a), A  $\rightarrow$  D or B  $\rightarrow$  C.)

The subscripts for  $\mu$  and  $v$  refer to the two concentration sets which are connected by the sharp boundary (e.g., the points B and C in Fig. 4 (a)). The knowledge of the characteristics  $\mu$  and  $v$  thus enables us to derive, with the help of eqn. (28), (29), (34) and (35), the form and rate of movement of all boundaries in the column or in the eluate.

Returning to the families of characteristics in the  $c_1 - c_2$  diagram, we see that the four cases mentioned in eqn. (28), (29), (34) and (35) do not describe all the possibilities which can occur if one solution is eluted by another. The two solutions following each other in these cases were exceptional in so far as both concentration sets lie on one characteristic. This, however, is not usually the case. If the two solutions following each other have no characteristic in common, and this is the rule, a new concentration plateau develops corresponding to that point of intersection of two charac-

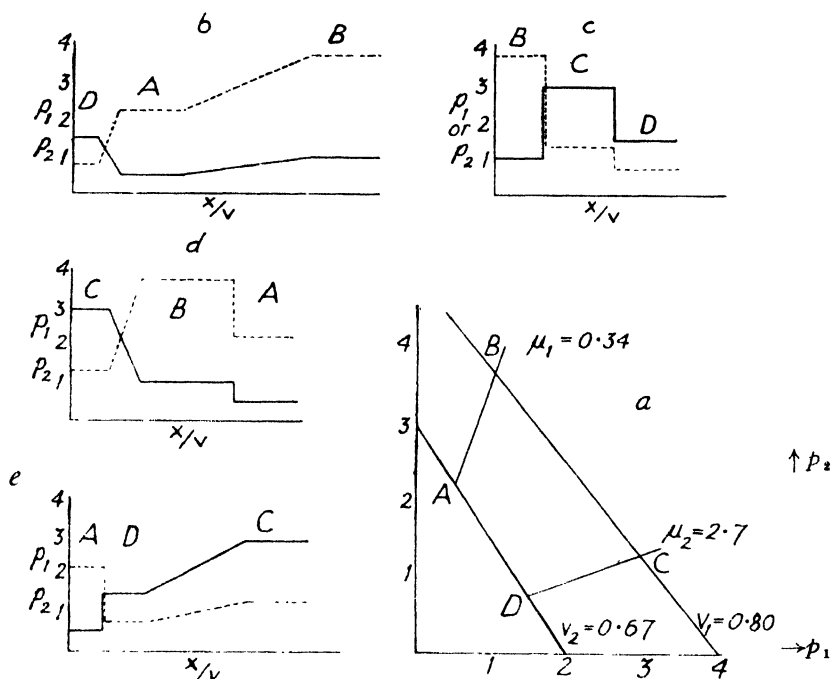


FIG. 4.—(a) Diagram of characteristics connecting two binary solutions, e.g., A and C, or B and D.  $\mu$  and  $\nu$  are the values of the gradients  $[dc_1/dc_2]$  of the characteristics. Concentration changes of the two solutes (solute I ———, solute II - - - -) in a column produced when

- (b) Solution D elutes a solution B.  
 (c) " B " " " D.  
 (d) " C " " " A.  
 (e) " A " " " C.

teristics passing through the two points which is stable according to Rule 1, which states that the positive characteristic always moves in front of a negative one. This means that we can have the following four cases, which can be classified only according to the variation of  $\mu$  and  $\nu$  along the column:

Case	$\mu$	$\nu$	Reference for Fig. 4 (a)
I	decreasing	decreasing	D $\rightarrow$ B (see Fig. 4 (b))
II	increasing	increasing	B $\rightarrow$ D (see Fig. 4 (c))
III	decreasing	increasing	C $\rightarrow$ A (see Fig. 4 (d))
IV	increasing	decreasing	A $\rightarrow$ C (see Fig. 4 (e))

The resulting boundaries of the column chromatograms with their spontaneously developing concentration plateaux are shown in Fig. 4 (b)–(e). The rate of movement, i.e., relative position of the boundaries, has been obtained from the  $\mu$ - and  $\nu$ -values by means of eqn. (28), (29), (34) and (35).

It should be pointed out that, while normally the sharp boundaries do not follow exactly the curves of the characteristics, they do so in the case of Langmuir isotherms, because here, due to the linearity of the characteristics,  $dc_1/dc_2$  is identical with  $\Delta c_1/\Delta c_2$ .

**Formation and Development of a Two Solute Band.**—During the formation of the original band, produced by feeding a solution  $c_1^\circ, c_2^\circ$  into an empty column ( $c_1 = 0, c_2 = 0$ ), we have clearly a case of type II (Fig. 4 (c)) with increasing  $\mu$  and  $\nu$ . An example is shown in Fig. 5 (a), the stable boundary course being represented  $S \rightarrow Q \rightarrow O$ . A new concentration plateau is created at  $Q$ , representing the pure frontal band of solute I (Fig. 5 (b)).

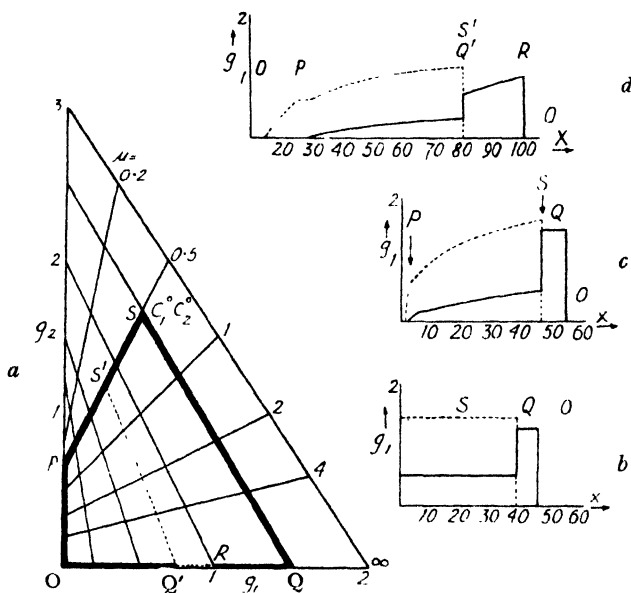


FIG. 5.—Formation and solvent-development of a chromatographic band of the original concentrations  $c_1^\circ, c_2^\circ$ .

(a) As shown in the diagram of  $g_1$ - $g_2$  characteristics.

Distribution of solute in a column for the above case.

(b) Original band.

(c) and (d) Stages in the development of the band.

If, after the formation of the original band, we proceed to "develop" the chromatogram by the addition of further amounts  $\nu$  of pure solvent ( $c_1 = 0, c_2 = 0$ ), we form a rear boundary of the type I (Fig. 4 (b)) with decreasing  $\mu$  and  $\nu$ , which in Fig. 5 (a) is represented by  $O \rightarrow P \rightarrow S$ .

This rear boundary forms a new concentration plateau, characterized by the point  $P$  in the  $f_1$ - $f_2$  (or  $g_1$ - $g_2$ ) diagram, which is the point at which  $f_1$  becomes zero (Fig. 5 (a) and (c)). The actual movements of the various points with continuing development ( $\nu$ ) follow directly from the application of the equations given in Tables I to V.<sup>6</sup>

Fig. 5 (a) gives the  $f_1$ - $f_2$  characteristics, which for a Langmuir isotherm are identical in form with the  $p_1$ - $p_2$  characteristics (eqn. (21a)), if one chooses the parameters,

$$g_1 = \frac{\beta b_1}{b_2 - b_1} \cdot f_1, \quad . \quad . \quad . \quad . \quad (36)$$

and 
$$g_2 = \frac{\beta b_2}{b_2 - b_1} \cdot f_2. \quad . \quad . \quad . \quad . \quad (37)$$

The equations of motion as function of the parameters  $\varphi = \left[ \frac{\partial f_1}{\partial f_2} \right]_{\psi}$  for the positive and  $\psi = \left[ \frac{\partial f_1}{\partial f_2} \right]_{\varphi}$  for the negative characteristics are similar to the eqn. (28), (29), (34) and (35).

We obtain :

$$\begin{bmatrix} \Delta x \\ \Delta v \end{bmatrix}_{\varphi} = \frac{1}{a_2 K} \left( \frac{1 + \varphi}{\frac{1}{K} + \varphi} \right) \left( \frac{1 + \psi}{\frac{1}{K} + \psi} \right)^2, \quad . \quad . \quad . \quad (38)$$

$$\begin{bmatrix} \Delta x \\ \Delta v \end{bmatrix}_{\psi} = \frac{1}{a_2 K} \left( \frac{1 + \varphi}{\frac{1}{K} + \varphi} \right)^2 \cdot \left( \frac{1 + \psi}{\frac{1}{K} + \psi} \right), \quad . \quad . \quad . \quad (39)$$

$$\begin{bmatrix} \Delta x \\ \Delta v \end{bmatrix}_{\varphi}^{\text{s.b.}} = \frac{1}{a_2 K} \left( \frac{1 + \varphi}{\frac{1}{K} + \varphi} \right) \left( \frac{1 + \psi_1}{\frac{1}{K} + \psi_1} \right) \left( \frac{1 + \psi_2}{\frac{1}{K} + \psi_2} \right), \quad . \quad . \quad . \quad (40)$$

$$\begin{bmatrix} \Delta x \\ \Delta v \end{bmatrix}_{\psi}^{\text{s.b.}} = \frac{1}{a_2 K} \left( \frac{1 + \varphi_1}{\frac{1}{K} + \varphi_1} \right) \left( \frac{1 + \varphi_2}{\frac{1}{K} + \varphi_2} \right) \left( \frac{1 + \psi}{\frac{1}{K} + \psi} \right) \quad . \quad . \quad . \quad (41)^*$$

For the construction of bands in the column, these  $f_1$ - $f_2$  characteristics are somewhat more convenient, as the areas under the  $x$ - $f$  curves (or  $x$ - $g$  curves) represent the original masses of the solutes which must be constant. (When dealing with elution ( $c$ - $v$ ) curves the advantage is with the  $c_1$ - $c_2$  characteristics, for the same reason.)

Returning to our Rule 1, we expect that the plateaux of P and Q (Fig. 5) should be stable and growing, as here positive precedes negative characteristic. However, at the point S this situation is reversed and this plateau, which had been experimentally introduced, prior to the development with pure solvent, is therefore unstable and constantly diminishing. Eventually the point S will disappear altogether, thereby making Q also unstable, so that both plateaux S and Q will eventually disappear. S slowly recedes on the line SP towards P; so we arrive at the diagram shown in Fig. 5 (a) by the sequence  $O \rightarrow P \rightarrow S' \rightarrow Q' \rightarrow R \rightarrow O$ , which results in the chromatogram shown in Fig. 5 (d), which type persists until the constantly receding point S' has merged with P, when Q' coincides with O, i.e., when separation is complete.

**Displacement Development.**—The use of the characteristics does not only apply to ordinary solvent development, but also to development with a solution containing a third solute. A particularly simple case arises when

\* Due to the fact that  $\beta(f_1 + f_2) < 1$ , it follows that  $\frac{1}{K} > -\psi$ .

the third solute is most strongly adsorbed, which leads to the so-called displacement development. In this case we get no ternary mixtures and we can, therefore, describe the situation by two adjoining maps of characteristics shown in Fig. 6. The original concentrations ( $c_1^0$ ,  $c_2^0$ ) are represented by the point E, the concentration of the developing solute ( $c_3^0$ ) by point A. The connection between A and E, and E and O under Rule 1, goes via the points B, D and F, all of which form stable plateaux in the chromatogram, while the original plateau E, where a negative characteristic precedes the positive one, is unstable and diminishes in length (Fig. 7 (a) and (b)). Eventually E will disappear completely and henceforward the path from D to O goes via the point G (Fig. 7 (c)). This change makes the previously formed plateaux D and F unstable, and they eventually disappear. The end of the chromatographic separation is then reached, leaving the plateaux A, B and G stable, all of which represent pure species of solute (Fig. 7 (d)).

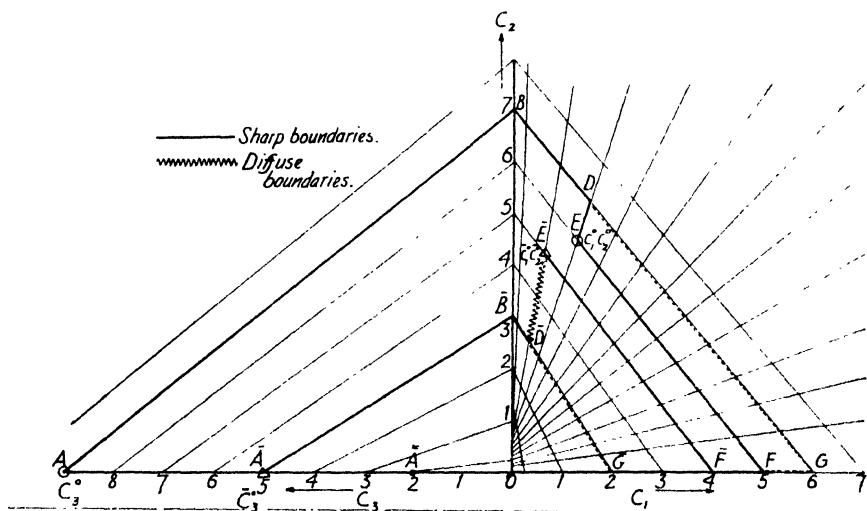


FIG. 6.—Development of a binary band with a solution containing a third solute, as shown in the diagram of  $c_1$ - $c_2$  and  $c_2$ - $c_3$  characteristics.

Example:  $b_1 = \frac{1}{2}$ ,  $b_2 = 1$ ,  $b_3 = 2$ .

We can immediately see from Fig. 6 what would happen if the developing concentration  $c_3^0$ , ( $\bar{A}$ ), is not high enough. In this case a diffuse boundary arises between the plateaux  $\bar{D}$  and  $\bar{E}$  (see also Fig. 6) which disappears eventually due to the instability of plateau  $\bar{E}$  and the separation ends with the plateaux  $\bar{A}$ ,  $\bar{B}$ ,  $\bar{G}$ .

Furthermore, if  $c_3^0$  is smaller than indicated by the point  $\bar{A}$  where the enveloping parabola (not shown) touches the abscissa, the only way from  $\bar{A}$  to E leads via O, which means that the third solute fails to make contact with the other solutes and then there is no longer any displacement development.

Actually, all these cases have already been discussed individually,<sup>4</sup> but the use of the "characteristics" makes it possible to give a unified picture of all possible chromatographic processes involving the separation of two solutes.

**Separation of Three Solutes.**—With a certain amount of algebraical discomfort, the theory can be extended to ternary solutions. We obtain then a three-dimensional system of characteristics, and changes caused by solvent development proceed by variation of one characteristic, while keeping the other two constant. However, as no new chromatographic features are derived from this extension, its discussion may well be postponed.

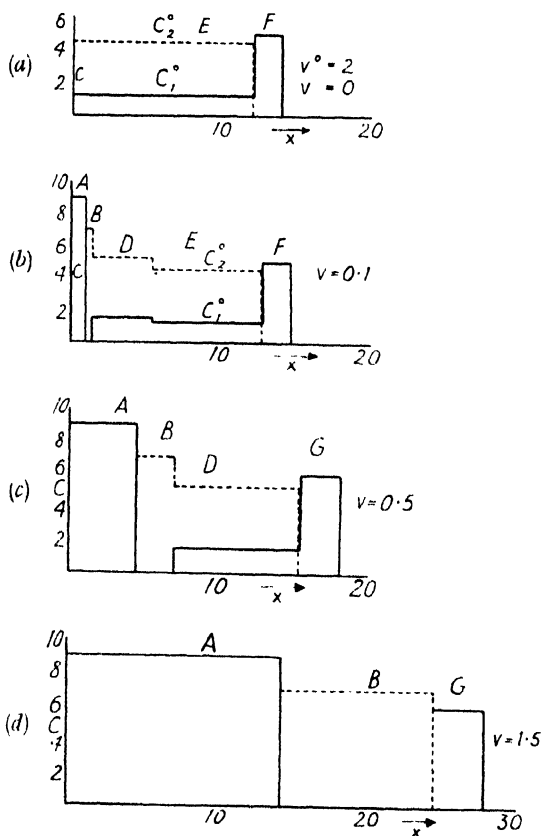


FIG. 7.—Concentration diagram in the column, deduced from Fig. 6, line A-B-D-E-F-O, etc.

(a) Original two solute band (solute I —, solute II - - -).

(b) (c) Stages in the development with a third solute.

(d) Complete separation.

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# CHROMATOGRAPHY WITH SEVERAL SOLUTES

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The theory of chromatography of a single solute has been given previously by de Vault and by Weiss. The present investigation has for its object a theory of chromatography for two or more solutes.

The treatment is based on the differential equations:

$$\frac{\partial c_1}{\partial x} + \frac{\partial q_1}{\partial v} = 0, \quad \frac{\partial c_2}{\partial x} + \frac{\partial q_2}{\partial v} = 0$$

connecting the concentrations  $c_1$  and  $c_2$  with the amounts adsorbed per unit length  $q_1$  and  $q_2$  respectively under equilibrium conditions, where  $v$  is the volume and  $x$  the distance along the column. We first investigate under what initial conditions these equations will have a solution which is a function of  $v/x$  and using this result determine solutions in a few cases.

We find that two very different phenomena may result. (i) In one case definite bands are formed in which the concentrations are constant. These constant values depend on the original bands and they can be determined in terms of the initial concentrations. (ii) In other cases bands of varying concentrations are formed. A typical example of the first type occurs when a uniform band of one or more solutes is developed with a solution of a different solute which is more strongly adsorbed than any of the solutes present initially (as illustrated, for instance, by Tiselius' displacement method).

The second type of chromatogram may occur when a band of two solutes at constant concentrations is developed with pure solvent or when a band consisting of a single solute is developed with a solution of a second solute which is much less strongly adsorbed than the first solute. Several examples are discussed and various criteria for distinguishing these cases are given.

The theory of chromatography of a single solute has been given by de Vault,<sup>1</sup> and by Weiss.<sup>2</sup> Subsequently a treatment for two or more solutes was briefly indicated by the present writers<sup>3</sup> and in some special cases was given by a number of authors.<sup>4</sup>

The object of this paper is to give a general theory for the chromatography of two or more solutes. We begin with a general discussion and then give examples for two and three solutes.

**I. General Discussion.**—The amounts adsorbed per unit cross-section at the level  $x$  of the band after the passing of a volume  $v$  are denoted by  $q_1(x, v)$  and  $q_2(x, v)$ . It is assumed that  $q_1$  and  $q_2$  depend only on the concentrations  $c_1$  and  $c_2$  of the solutes (1) and (2) and that the adsorption equilibrium is practically always established.

If  $q_1$ ,  $q_2$ ,  $c_1$  and  $c_2$  are continuous functions with continuous partial derivatives, considerations of conservation of mass imply that:

$$\frac{\partial c_1}{\partial x} + \frac{\partial q_1}{\partial v} = 0 \quad . \quad . \quad . \quad . \quad (1.1)$$

and

$$\frac{\partial c_2}{\partial x} + \frac{\partial q_2}{\partial v} = 0 \quad . \quad . \quad . \quad . \quad (1.2)$$

<sup>1</sup> De Vault, *J. Amer. Chem. Soc.*, 1943, **65**, 532; also Wilson, *ibid.*, 1940, **62**, 1583.

<sup>2</sup> Weiss, *J. Chem. Soc.*, 1943, 297.

<sup>3</sup> Offord and Weiss, *Nature*, 1945, **155**, 725.

<sup>4</sup> Cf. Glueckauf, *Proc. Roy. Soc. A*, 1946, **186**, 35; *J. Chem. Soc.*, 1947, 1308, 1321.

However, we shall not assume that  $c_1$  and  $c_2$  are continuous functions of  $x$  and  $v$ . This is based on results obtained by one of us and by others<sup>1,2</sup> in the discussion of the chromatography of a single solute. At first sight it may seem somewhat artificial to admit discontinuous functions, but it must be borne in mind that  $c_1$  and  $c_2$  represent certain mathematical functions which satisfy eqn. (1.1) and (1.2). These functions will, in general, represent the concentrations only approximately because they were derived under the assumption of equilibrium conditions and with the neglect of any diffusion and convection effects. Thus though the concentrations may be continuous, it may well be that they can be best approximated by discontinuous functions. Experience in the chromatography of a single solute shows that this is actually the case.

Let us suppose now that there is a certain front of discontinuity AB. Let  $q_{1(I)}$  and  $q_{2(I)}$  be the amounts adsorbed per unit length in the band immediately above the level AB and  $q_{1(II)}$  and  $q_{2(II)}$  be the corresponding quantities in the band immediately below AB. We shall suppose that the concentrations remain constant as the level AB moves down the tube. Let  $c_{1(I)}$ ,  $c_{2(I)}$ ,  $c_{1(II)}$  and  $c_{2(II)}$  be the corresponding concentrations. Suppose that as the result of the addition of a volume  $\delta v$  the level of discontinuity moves down the tube a distance  $\delta x$  to a level A'B'. (see Fig. 1).

Then the decrease in the amounts of the substances (1) and (2) in the section (AB)(B'A') will be

$$\left\{ q_{1(II)} - q_{1(I)} \right\} \delta x \text{ and } \left\{ q_{2(II)} - q_{2(I)} \right\} \delta x,$$

and these quantities will be equal to the *increases* in the amounts of solute in the small volume  $\delta v$  which are

$$\left\{ c_{1(II)} - c_{1(I)} \right\} \delta v \text{ and } \left\{ c_{2(II)} - c_{2(I)} \right\} \delta v$$

respectively, and hence

$$\left\{ q_{1(II)} - q_{1(I)} \right\} \delta x - \left\{ c_{1(II)} - c_{1(I)} \right\} \delta v = 0, \quad (2.1)$$

$$\left\{ q_{2(II)} - q_{2(I)} \right\} \delta x - \left\{ c_{2(II)} - c_{2(I)} \right\} \delta v = 0. \quad (2.2)$$

Since  $\delta v$  and  $\delta x$  are not zero and since these equations must hold simultaneously, we get

$$\frac{\delta v}{\delta x} = \frac{q_{1(II)} - q_{1(I)}}{c_{1(II)} - c_{1(I)}} = \frac{q_{2(II)} - q_{2(I)}}{c_{2(II)} - c_{2(I)}}. \quad (2.3)$$

Allowing  $\delta x$  and  $\delta v$  to tend to zero, we deduce that

$$\frac{dv}{dx} = \frac{q_{1(II)} - q_{1(I)}}{c_{1(II)} - c_{1(I)}} = \frac{q_{2(II)} - q_{2(I)}}{c_{2(II)} - c_{2(I)}}. \quad (3)$$

This is a differential equation giving the rate at which a level of discontinuity AB moves down the tube, in terms of the ratio of the discontinuities in the amounts adsorbed and the concentrations.

We shall now make some general assumptions concerning  $q_1$  and  $q_2$ . We shall suppose that they depend only on  $c_1$  and  $c_2$  and that they are regular functions of  $c_1$  and  $c_2$ ; that is to say, that they can be expanded in a Taylor series in  $c_1$  and  $c_2$ . Thus,

$$q_1 = \sum_{m=0}^{\infty} \sum_{n=0}^{\infty} a_{m,n} c_1^m c_2^n, \quad q_2 = \sum_{m=0}^{\infty} \sum_{n=0}^{\infty} b_{m,n} c_1^m c_2^n.$$



Since obviously  $q_1$  is zero if  $c_1$  is zero and  $q_2$  is zero if  $c_2$  is zero, we must have  $a_{0,n} = b_{m,0} = 0$  for all  $m$  and  $n$ .

This implies that

$$\left(\frac{\partial q_1}{\partial c_2}\right)_{c_1=0} = \left(\frac{\partial q_2}{\partial c_1}\right)_{c_2=0} = 0 \quad (4)$$

Thus the eqn. (I) become

$$\frac{\partial c_1}{\partial x} + \frac{\partial q_1}{\partial c_1} \frac{\partial c_1}{\partial v} + \frac{\partial q_1}{\partial c_2} \frac{\partial c_2}{\partial v} = 0, \quad (5.1)$$

$$\frac{\partial c_2}{\partial x} + \frac{\partial q_2}{\partial c_1} \frac{\partial c_1}{\partial v} + \frac{\partial q_2}{\partial c_2} \frac{\partial c_2}{\partial v} = 0. \quad (5.2)$$

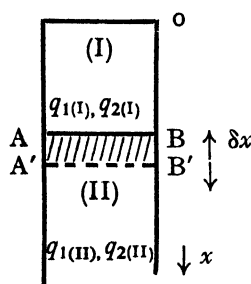


FIG. 1.—Front of discontinuity AB separating the regions (I) and (II).

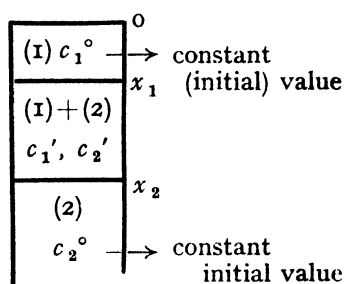


FIG. 3.—Uniformly distributed solute (2) partly developed with a solution of a more strongly adsorbed substance (1).

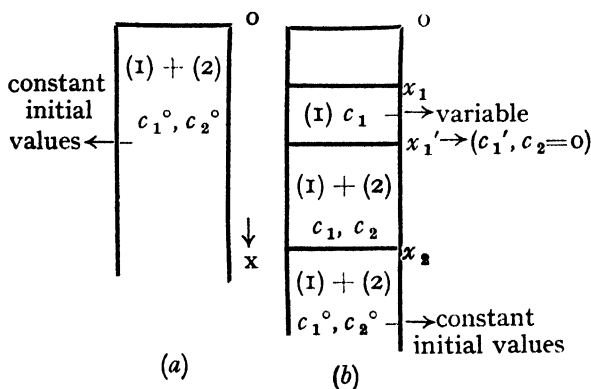


FIG. 2.—(a) 2 Solutes: initial band.  
(b) 2 Solutes: after partial development.

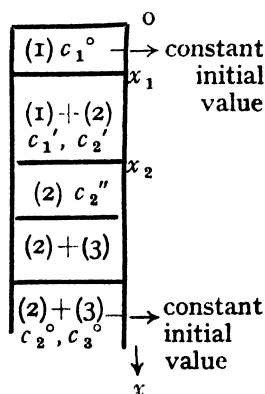


FIG. 4.—Originally uniform mixture of two substances (2) and (3) partly developed with a solution of a third substance (1) which is more strongly adsorbed.

One could solve any problem involving two solutes and without discontinuities if one knew a solution of these equations under sufficiently general conditions. However, no such solution is known. The classical

solution\* assumes that  $c_1$  and  $c_2$  are regular functions of  $x$  and  $v$  and this hypothesis is difficult to apply, since the initial conditions as usually stated in nearly every problem in chromatography require  $c_1$  and  $c_2$  to be discontinuous for  $x=0$  and  $v=0$ .

There is, however, an important class of problems where it is possible to obtain solutions of these equations. It can be shown that under certain very special initial conditions the solutions of these equations will be approximately functions of  $(v/x)$ . The initial conditions for this are that when  $x=0$ ,  $c_1$  and  $c_2$  are both constant for all  $v$  and that when  $v=0$  the distributions  $q_1$  and  $q_2$  of solute in the tube are constant for all  $x$ .

Now it happens that usually under the experimental conditions in chromatography these conditions are in fact often satisfied to a first approximation. For instance, they are fulfilled when an (infinite) band containing adsorbed material from a solution of two solutes of constant concentrations is developed with pure solvent or with a solution of a third substance. In practice where the columns are of finite length it is reasonable to expect that the subsequent development would be the same since it is determined by the state of affairs at the top of the chromatogram. It would therefore seem that a solution of eqn. (5) under such initial conditions would furnish all that a practical chromatographer would desire. However, another difficulty now arises. It appears that under such initial conditions eqn. (5) need not have a unique solution and so no mathematical argument can be fully conclusive and any results obtained will have to be tested by experiment.

Let us proceed with the discussion of eqn. (5) on these lines. Substituting  $y=(v/x)$ , and assuming that  $c_1$  and  $c_2$  depend on  $y$  as defined above, one gets

$$\left(\frac{\partial q_1}{\partial c_1} - y\right) \frac{dc_1}{dy} + \frac{\partial q_1}{\partial c_2} \frac{dc_2}{dy} = 0, \quad (6.1)$$

$$\frac{\partial q_2}{\partial c_1} \frac{dc_1}{dy} + \left(\frac{\partial q_2}{\partial c_2} - y\right) \frac{dc_2}{dy} = 0. \quad (6.2)$$

In general, the solution of these equations will be

$$\frac{dc_1}{dy} = \frac{dc_2}{dy} = 0.$$

The necessary and sufficient conditions for them to have a solution other than this one is

$$\begin{vmatrix} \left(\frac{\partial q_1}{\partial c_1} - y\right) & \frac{\partial q_1}{\partial c_2} \\ \frac{\partial q_2}{\partial c_1} & \left(\frac{\partial q_2}{\partial c_2} - y\right) \end{vmatrix} = 0. \quad (7.1)$$

This corresponds to the quadratic equation

$$y^2 - y \left( \frac{\partial q_1}{\partial c_1} + \frac{\partial q_2}{\partial c_2} \right) + \frac{\partial q_1}{\partial c_1} \frac{\partial q_2}{\partial c_2} - \frac{\partial q_1}{\partial c_2} \frac{\partial q_2}{\partial c_1} = 0, \quad (7.2)$$

which has the roots

$$\alpha(c_1, c_2) = \frac{1}{2} \left[ \left( \frac{\partial q_1}{\partial c_1} + \frac{\partial q_2}{\partial c_2} \right) + \left\{ \left( \frac{\partial q_1}{\partial c_1} - \frac{\partial q_2}{\partial c_2} \right)^2 + 4 \frac{\partial q_1}{\partial c_2} \frac{\partial q_2}{\partial c_1} \right\}^{\frac{1}{2}} \right], \quad (8.1)$$

$$\beta(c_1, c_2) = \frac{1}{2} \left[ \left( \frac{\partial q_1}{\partial c_1} + \frac{\partial q_2}{\partial c_2} \right) - \left\{ \left( \frac{\partial q_1}{\partial c_1} - \frac{\partial q_2}{\partial c_2} \right)^2 + 4 \frac{\partial q_1}{\partial c_2} \frac{\partial q_2}{\partial c_1} \right\}^{\frac{1}{2}} \right], \quad (8.2)$$

\* See, for example, Goursat, *Leçons sur l'intégration des équations aux dérivées partielles du premier ordre* (1891), p. 2.

and thus eqn. (7) may be written

$$(y-\alpha)(y-\beta) = 0. \quad (9)$$

Whenever (7) is satisfied (6) gives

$$\frac{dc_2/dy}{dc_1/dy} = \frac{(y-\partial q_1/\partial c_1)}{\partial q_1/\partial c_2},$$

that is,

$$\frac{dc_2}{dc_1} = \frac{(\alpha - \partial q_1/\partial c_1)}{(\partial q_1/\partial c_2)}. \quad (10.1)$$

or

$$\frac{dc_2}{dc_1} = \frac{(\beta - \partial q_1/\partial c_1)}{(\partial q_1/\partial c_2)}. \quad (10.2)$$

according to which solution of (7) we take. In either case,

$$\alpha = dq_1/dc_1 \text{ and } \beta = dq_1/dc_1 \quad (11)$$

and similarly

$$\alpha = dq_2/dc_2,$$

so that in a *variable* band we have

$$dq_1/dc_1 = dq_2/dc_2. \quad (12)$$

## II Examples

**(i) Chromatogram formed from two solutes and developed with pure solvent.**—As we have already remarked our special conditions are satisfied in this case and we may apply the general theory developed above.

Let us first consider the problem that arises when a solution of two solutes is poured on to a column of adsorbent material, which is initially free of solutes. The first question to be decided is: *can a variable band be found in these circumstances?* If this does occur then eqn. (10.1) and (10.2) hold throughout the band. Moreover it is clear that  $dc_2/dc_1$  will be positive since  $c_1$  and  $c_2$  will either increase together or decrease together. Further, it is to be expected that the presence of one solute will hinder the adsorption of the others so that  $\partial q_1/\partial c_2$  and  $\partial q_2/\partial c_1$  will be negative and their product  $(\partial q_1/\partial c_2)(\partial q_2/\partial c_1)$  positive. Hence from the eqn. (8) one obtains for the discriminant:

$$\left\{ \left( \frac{\partial q_1}{\partial c_1} - \frac{\partial q_2}{\partial c_2} \right)^2 + 4 \frac{\partial q_1}{\partial c_2} \frac{\partial q_2}{\partial c_1} \right\}^{\frac{1}{2}} > \left| \frac{\partial q_2}{\partial c_1} - \frac{\partial q_2}{\partial c_2} \right| \quad (13)$$

and so it follows from eqn. (8) that  $(\alpha - \partial q_1/\partial c_1)$  is *positive* and  $(\beta - \partial q_1/\partial c_1)$  is *negative*. Consequently, of the two eqn. (10), (10.1) gives a negative value for  $dc_2/dc_1$  and (10.2) a positive value. Therefore, throughout this band we must have

$$\frac{dc_2}{dc_1} = \frac{(\beta - \partial q_1/\partial c_1)}{\partial q_1/\partial c_2}, \quad (14.1)$$

or

$$\beta = \frac{\partial q_1}{\partial c_1} + \frac{\partial q_1}{\partial c_2} \frac{dc_2}{dc_1} = \frac{dq_1}{dc_1}. \quad (14.2)$$

But then (9) implies that

$$y = v/x = \beta, \quad (15.1)$$

or

$$v/x = dq_1/dc_1. \quad (15.2)$$

If now the level  $x$  is fixed and the volume  $v$  increased,  $c_1$  and  $c_2$  will increase. But with normal isotherms  $dq_1/dc_1$  is a *decreasing* function of  $c_1$  (i.e., with all ordinary adsorption isotherms as  $c_1$  increases  $dq_1/dc_1$  decreases) and so

low concentrations is not greatly affected by the presence of solute (1) in the solution. If  $z_1$  and  $z_2$  decrease, but  $z_1$  decreases more rapidly than  $z_2$ , there will be a point at which  $z_1 = z_2$  (since initially  $z_1$  is the larger). That is a  $c_1$  for which eqn. (22) is satisfied. Hence, in this case, when the column consisting of solutes (1) and (2) corresponding to constant concentrations  $c_1^0$  and  $c_2^0$  (Fig. 2 (a)) is developed with a volume  $v$  of solvent at the top of the tube there will be a *clear band* (from 0 to  $x_1$ , Fig. 2 (b)).

If  $(\partial q_1 / \partial c_1)_{c_1=c_2^0} > (\partial q_2 / \partial c_2)_{c_1=c_2^0}$ , then at a level  $x_1$ , given by

$$v/x_1' = (\partial q_1 / \partial c_1)_{c_1=c_2^0}, \quad (24)$$

a band of solute (1) will occur the concentration at any level  $x$  (in the region from  $x_1$  to  $x_1'$ , Fig. 2 (b)) being given by

$$v/x = (\partial q_1 / \partial c_1)_{c_2^0}, \quad (25)$$

the concentration  $c_1$  of solute (1) within this band increases until it reaches a critical value  $c_1'$ , given by eqn. (22).

After this, there follows a mixed band ( $x_1'$  to  $x_2$ ) where the concentrations  $c_1$  and  $c_2$  are connected by the differential eqn. (12) subject to the conditions that when  $c_1 = c_1'$ ,  $c_2 = 0$ . This development continues to a certain level where a sharp change in the concentrations occurs and we arrive at the undeveloped point of the band with the concentrations at their original values of  $c_1^0$  and  $c_2^0$  (Fig. 2 (b)).

Various other solutions may be possible in this case, depending on the characters of the isotherms, but the above solution appears to be of particular interest.

There is one further point of some importance. The chromatogram we have described is that obtained when an infinite band is developed. In practice the band will be of finite length of mixed solutes (1) and (2) followed usually by a band of the less strongly adsorbed solute (2). On developing with (pure) solvent the nature of the development at the top of the tube is clearly independent of the length of the band. The solution we have given then enables us to calculate the volume of solvent required to obtain complete separation. Writing

$$\partial(c_1) = q_1(c_1, 0),$$

then the amount of solute (1) in the band between the levels  $x_1$  and  $x_1'$  (see Fig. 2 (b)) is

$$\int_{x_1'}^{x_1} \partial(c_1) dx = \int_{c_1'}^{c_1^0} \partial(c_1) \frac{dx}{dc_1}, \quad (26.1)$$

and on integration by parts this becomes

$$\left[ \partial(c_1) x \right]_{c_1'}^{c_1^0} - \int_{c_1'}^{c_1^0} x \partial'(c_1) dc_1 = x_1' \partial(c_1') - \int_{c_1'}^0 x \partial'(c_1) dx,$$

bearing in mind that  $\partial(0) = 0$ .

Now throughout this band  $\frac{x}{v} = \partial'(c_1)$ , where  $v$  the volume added is, of course, a constant, and so the above expression becomes

$$v \left\{ \frac{\partial(c_1')}{\partial(c_1')} - c_1' \right\}. \quad (26.2)$$

consequently the above equation can never be satisfied. Hence in this case there can be no variable band.

(ii) Development of a column consisting of a uniform mixture of two solutes (1) and (2) with pure solvent.—We shall assume that when development occurs it will be a *continuous* phenomenon and so throughout the developed bands of the two solutes eqn. (7) holds. Let us now suppose that

$$(16) \quad \left( \frac{\partial q_1}{\partial c_1} \right)_{c_1=c_1^0, c_2=0} < \left( \frac{\partial q_2}{\partial c_2} \right)_{c_1=0, c_2=c_2^0}$$

This corresponds to treating the solute (1) as the more strongly adsorbed one. Then from eqn. (4) and (8) one has

$$\alpha(c_1, 0) = \left( \frac{\partial q_1}{\partial c_1} \right)_{c_1=c_1^0, c_2=0} \quad \text{and} \quad \beta(c_1, 0) = \left( \frac{\partial q_2}{\partial c_2} \right)_{c_1=0, c_2=c_2^0}$$

and so (7) reduces to

$$(17) \quad \left\{ \gamma - \left( \frac{\partial q_1}{\partial c_1} \right)_{c_1=c_1^0, c_2=0} \right\} \left\{ \gamma - \left( \frac{\partial q_2}{\partial c_2} \right)_{c_1=0, c_2=c_2^0} \right\} = 0.$$

But (16) implies that for small  $c_1$ ,

$$(18) \quad \left( \frac{\partial q_1}{\partial c_1} \right)_{c_1=0, c_2=c_2^0} < \left( \frac{\partial q_2}{\partial c_2} \right)_{c_1=0, c_2=c_2^0},$$

and so since  $\gamma = (v/x)$  is a decreasing function of  $x$  (i.e., large for  $x$  small) we must have

$$(19) \quad (v/x) = \left( \frac{\partial q_1}{\partial c_1} \right)_{c_1=0, c_2=c_2^0}$$

On the other hand when  $c_2 > 0$ , clearly  $dc_2/dc_1$  is positive and so as above,

$$(20) \quad \frac{dc_2}{dx} = \frac{\left( \frac{\partial q_1}{\partial c_1} \right)_{c_1=0, c_2=c_2^0}}{\left( \beta - \left( \frac{\partial q_1}{\partial c_1} \right)_{c_1=0, c_2=c_2^0} \right)},$$

and this implies that

$$(21) \quad v/x = \beta,$$

or, in other words, that for  $c_2 > 0$  it is the second factor in (7) which vanishes, while for  $c_2 = 0$  it is the first factor. But by our hypothesis the development is a *continuous* phenomenon and this means that eqn. (7) must hold for *all*  $c_2$ . If now we imagine  $c_2$  tends to zero in (7), then since for  $c_2 > 0$  the second factor is zero and for  $c_2 = 0$  the first factor is zero, it follows that at the point where  $c_2$  just vanishes, both factors must be zero and so at this level,

$$(22) \quad \left( \frac{\partial q_1}{\partial c_1} \right)_{c_1=0, c_2=c_2^0} = \left( \frac{\partial q_2}{\partial c_2} \right)_{c_1=0, c_2=c_2^0}$$

Eqn. (22) determines the concentration  $c_1'$  of the solute (1) at the level at which  $c_2$  in zero and the level  $x_1'$  at which this occurs is given by

$$(23) \quad v/x_1' = \left( \frac{\partial q_1}{\partial c_1} \right)_{c_1=c_1', c_2=0} = \left( \frac{\partial q_2}{\partial c_2} \right)_{c_1=c_1', c_2=c_2^0}.$$

Let us examine eqn. (22). The two functions  $z_1 = \left( \frac{\partial q_1}{\partial c_1} \right)_{c_1=0, c_2=c_2^0}$  and  $z_2 = \left( \frac{\partial q_2}{\partial c_2} \right)_{c_1=0, c_2=c_2^0}$  are both usually decreasing functions of the same variable  $c_1$ .

The first function  $z_1$  denotes the rate of change of the amount adsorbed of solute (1) with concentration of this solute in the absence of solute (2). This quantity, for all ordinary isotherms, decreases as the concentration  $c_1$  increases. The second function  $z_2$  represents likewise the rate of change of adsorbed amount of solute (2) with concentration  $c_2$ , for very low values of  $c_2$  and in a solution which also contains the solute (1) at a certain concentration  $c_1$ . Thus the curves for  $z_1$  and  $z_2$  are essentially different in character:  $z_2$  will normally decrease as  $c_1$  increases but the decrease may be expected to be small compared with the corresponding decrease in  $z_1$ . This is the case if the "strength" at which solute (2) is adsorbed at very

Now suppose that the total amount of solute (I) present was  $m_1$ . Then, the tube being of unit cross-section, the volume  $v_s$  required to obtain total separation will be

$$v_s = m_1 \left\{ \frac{Q(c_1')}{Q'(c_1')} - c_1' \right\}^{-1}, \quad (27)$$

where  $c_1'$  is given by (22).

**(iii) An infinite column of uniformly distributed solute is developed with a solution of a more strongly adsorbed substance.**—Our simple equations are valid in this case. We shall suppose, as usual, that solute (I) is the more strongly adsorbed. In any mixed band  $dc_2/dc_1$  is negative and this implies that:

$$\frac{v}{x} = \alpha(c_1, c_2) = \frac{1}{2} \left[ \left( \frac{\partial q_1}{\partial c_1} + \frac{\partial q_2}{\partial c_2} \right) + \left\{ \left( \frac{\partial q_1}{\partial c_1} - \frac{\partial q_2}{\partial c_2} \right)^2 + 4 \frac{\partial q_1}{\partial c_2} \frac{\partial q_2}{\partial c_1} \right\}^{\frac{1}{2}} \right]. \quad (28)$$

$$\text{If } \left( \frac{\partial q_1}{\partial c_2} \right) \left( \frac{\partial q_2}{\partial c_1} \right)$$

is small relative to the remaining terms, this implies that

$$\frac{v}{x} = \frac{\partial q_1}{\partial c_1} + \frac{4 \left( \frac{\partial q_1}{\partial c_2} \right) \left( \frac{\partial q_2}{\partial c_1} \right)}{\left| \frac{\partial q_1}{\partial c_1} - \frac{\partial q_2}{\partial c_2} \right|}. \quad (29)$$

Suppose a volume  $v$  of a solution of solute (I) is added, then as we go down the tube  $(\partial q_1/\partial c_1)$  increases as  $c_1$  decreases. Also for  $c_2=0$ ,  $\partial q_1/\partial c_1$  is zero but not zero for  $c_2 > 0$ . Hence the second term also increases (the product  $(\partial q_1/\partial c_2)(\partial q_2/\partial c_1)$  being positive). Therefore both terms on the right-hand side increase which is impossible since with increasing  $x$  the left-hand side decreases. Hence we are led to a contradiction and must conclude that in this case there can be *no variable band*. Therefore the only solution possible is a *discontinuous* one. To satisfy eqn. (3) there must be *two discontinuities* separated by a mixed band. If  $c_1'$  and  $c_2'$  denote here the concentrations of the solutes in the mixed band then,

$$\frac{q_1(c_1^0, 0) - q_1(c_1', c_2')}{(c_1^0 - c_1')} = \frac{q_2(c_1', c_2')}{c_2'}, \quad (30.1)$$

and

$$\frac{q_1(c_1', c_2')}{c_1'} = \frac{q_2(0, c_2^0) - q_2(c_1', c_2')}{(c_2^0 - c_2')}. \quad (30.2)$$

are the two simultaneous equations for  $c_1'$  and  $c_2'$ . If  $x_1$  denotes the level of the top of the mixed band and  $x_2$  the level of the bottom (Fig. 3) after a volume  $v$  has been added, then by eqn. (3), since  $x=0$  when  $v=0$ ,

$$\frac{v}{x_1} = \frac{q_2(c_1', c_2')}{c_2'} \quad (31.1)$$

$$\frac{v}{x_2} = \frac{q_1(c_1', c_2')}{c_1'} \quad (31.2)$$

**(iv) Band consisting of a uniform mixture of two substances (2) and (3) which is developed with a solution of a third substance (1).**—In this case we have, in general, a cubic equation to solve and so a detailed analysis would be somewhat complicated. We shall consider only the case when the substance (I) is more strongly adsorbed than the other two substances and even then we shall only instance two cases which might arise.

(a) If the adsorption isotherms of the three substances are represented by  $q_1$ ,  $q_2$  and  $q_3$ , and if

$$\frac{q_1(c_1^0, 0, 0)}{c_1^0} > \left( \frac{\partial q_2}{\partial c_2} \right)_{c_1=c_3=c_2=0} > \left( \frac{\partial q_3}{\partial c_3} \right)_{c_1=c_3=c_2=0} \quad (32)$$

then there is no interaction between (1) and the mixture of the components (2) and (3). A band of solute (1) is formed at the top and the mixture (2) and (3) is developed as in Example (ii).

(b) If

$$\left( \frac{\partial q_2}{\partial c_2} \right)_{c_1=c_3=c_2=0} > \frac{q_1(c_1^0, 0, 0)}{c_1^0} > \left( \frac{\partial q_3}{\partial c_3} \right)_{c_1=c_3=c_2=0} \quad (33)$$

then at the top of the tube we shall have a chromatogram corresponding to Example (iii). Altogether there may be *five* bands. In the *top band* the substance (1) at concentration  $c_1^0$ . In the *second band* solutes (1) and (2) corresponding to concentrations  $c_1'$  and  $c_2'$ . In the *third band*, (2) at concentration  $c_2''$ . The *fourth band* may be a mixed band as in Example (i) and in the *fifth band*, (2) and (3) are present at their original concentrations  $c_2^0$  and  $c_3^0$  (see Fig. 4).

The values of  $c_1'$ ,  $c_2'$  and  $c_2''$  are given by the following equations:

$$\frac{q_1(c_1^0, 0, 0) - q_1(c_1', c_2', 0)}{(c_1^0 - c_1')} = \frac{q_2(c_1', c_2', 0)}{c_2'}, \quad (34.1)$$

$$\frac{q_1(c_1', c_2', 0)}{c_1'} = \frac{q_2(0, c_2'', 0) - q_2(c_1', c_2', 0)}{c_2'}, \quad (34.2)$$

$$\frac{q_2(0, c_2^0, c_3^0)}{(c_2^0 - c_2'')} = \frac{q_3(0, c_2^0, c_3^0)}{c_3^0}. \quad (34.3)$$

The levels  $x_1$  and  $x_2$  corresponding to the top and bottom of the first mixed band are then given by

$$\frac{v}{x_1} = \frac{q_2(c_1', c_2', 0)}{c_2'}. \quad (35.1)$$

$$\frac{v}{x_2} = \frac{q_1(c_1', c_2', 0)}{c_1'}. \quad (35.2)$$

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## THEORY OF FRONTAL ANALYSIS AND DISPLACEMENT DEVELOPMENT

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A simple theoretical model, which is useful for practical applications of chromatography, is discussed for frontal analysis and displacement development.

It can be said that the theory for chromatographic separation has been developed along two different lines. One group of workers has tried to use as simple theoretical models as possible in order to get simple but still useful results for the actual calculations when separating complex mixtures. Another

group of workers has tried to work out a very detailed theory for the very complex phenomena which take place in a column. Due to mathematical difficulties this type of work has mostly been carried out for systems with one or at most two solutes. Such results are usually of little use in practical applications of chromatography, but they are of utmost importance when one is trying to get a deeper understanding of the chromatographic process in general or to reach the limit of resolving power as in isotope separation.

In this paper the "simple" theory which is useful for practical applications of chromatography will be discussed for two rather important special cases, namely, frontal analysis and displacement development.

Frontal analysis, introduced by Tiselius<sup>1</sup> in 1940, is the simplest possible arrangement for chromatography. The solution containing one or several solutes is forced through the column with adsorbent, previously washed with pure solvent. The concentration of the effluent is determined and plotted as a function of the volume which has passed the filter. In this way characteristic curves are obtained showing one step for every solute. In Fig. 1 is seen a schematic curve for two solutes. The first step contains

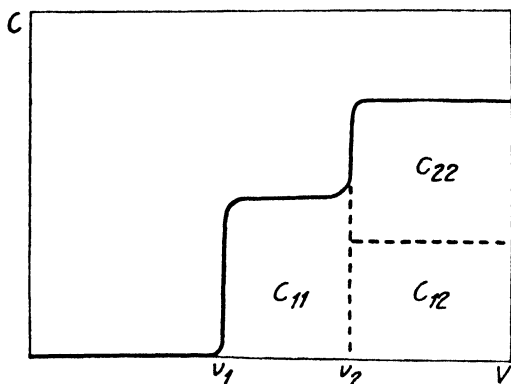


FIG. 1.

solute 1, the second step solute 1 and 2 and so on. The volume which has passed when a particular solute  $i$  breaks through is called the retention volume for component  $i$ . This volume includes the small volume necessary to replace the solvent in the column at the beginning of the experiment. After subtracting this small volume we get the corrected retention volume  $v_i$ . The corrected retention volume per gram adsorbent is called specific retention volume  $v_i^0$ .

Frontal analysis has the great advantage that the adsorbed substance does not have to be eluted again and this method can therefore be used even in the case of irreversible adsorption. That is of great value as it is often difficult to find adsorbents with sufficient selectivity which will give off the adsorbed substance quantitatively. The disadvantage is, however, that the heights of the steps are not proportional to the concentrations of the corresponding solutes in the original solution. The reason for that is adsorption displacement which means that when solute 2 is passing down the column and adsorbed, it will knock off some already adsorbed molecules of solute 1 from the adsorbent and consequently the concentration  $c_{11}$  of solute 1 in step 1 is higher than in the original solution. We introduce the general notation  $c_{i,j}$  for the concentration of solute  $i$  in step  $j$ .

<sup>1</sup> Tiselius, *Arkiv. Kemi, Min. Geol. B*, 1940, **14**, No. 22.



For quantitative work it is therefore necessary to calculate the composition of the solution from the observed retention volumes and the heights of the steps. In order to get simple results of practical value for systems with many solutes it is necessary to make simplified assumptions. We assume instantaneous equilibrium and perfectly plane fronts. The first treatment along these lines was given by de Vault<sup>2</sup> and further discussed by the present author.<sup>3, 4</sup>

In the following we will restrict the discussion to systems with only two solutes. Extension of the theory to an arbitrary number of solutes is quite simple<sup>2-4</sup> but will not bring forward anything new in principle.

For a system with only one solute of concentration  $c_{11}$  the amount adsorbed  $a_1^0 = f_1(c_{11})$  per gram adsorbent in the column is evidently  $v_1^0 c_{11}$  and we have

$$v_1^0 = \frac{f_1(c_{11})}{c_{11}} \quad . \quad . \quad . \quad . \quad . \quad . \quad (1)$$

For two solutes we get in the same way:

$$a_1^0 = f_1(c_{12}, c_{22}) = v_2^0 c_{12} - (v_2^0 - v_1^0) c_{11}, \quad . \quad . \quad . \quad (2)$$

$$a_2^0 = f_2(c_{12}, c_{22}) = v_2^0 c_{22}. \quad . \quad . \quad . \quad (3)$$

If we now introduce a suitable equation for the adsorption isotherm these expressions can be simplified considerably. The simplest results are obtained from Langmuir's isotherm

$$a_1^0 = k_1 c_{12} / (1 + l_1 c_{12} + l_2 c_{22}), \quad . \quad . \quad . \quad (4)$$

$$a_2^0 = k_2 c_{22} / (1 + l_1 c_{12} + l_2 c_{22}). \quad . \quad . \quad . \quad (5)$$

By dividing eqn. (2) and (3) we get

$$c_{12} = c_{11} \left( \frac{1 - \frac{v_1}{v_2}}{1 - \frac{k_1}{k_2}} \right) \quad . \quad . \quad . \quad . \quad . \quad . \quad (6)$$

We have thus obtained a very simple formula for the calculation of the correct concentration  $c_{12}$  from the observed value  $c_{11}$ . It should be noted that  $k_i$  is the retention volume for solute  $i$  at infinite dilution (from eqn. (1)).

In case of several solutes eqn. (6) will take the form

$$c_{i(m+1)} = c_{im} \frac{1 - \frac{k_i}{k_m} \cdot \frac{v_m}{v_{m+1}}}{1 - \frac{k_i}{k_{m+1}}} \quad . \quad . \quad . \quad (7)$$

It is consequently possible to calculate all concentrations in this way provided that we know the  $k$  values, which have to be determined from experiments with pure components or by other suitable methods.

It must be borne in mind, however, that this displacement effect is not primarily dependent on the actual displacement process in the column. Changes of concentration of this type will occur in all systems where a separation is caused by differences in mobility between the different solutes and where these mobilities depend on the concentrations. These "displacement" effects which are a consequence of the conservation of the mass of the solute are also well known both in electrophoresis<sup>5</sup> and in

<sup>2</sup> De Vault, *J. Amer. Chem. Soc.*, 1943, **65**, 532.

<sup>3</sup> Claesson, *Arkiv. Kemi, Min. Geol. A*, 1946, **23**, No. 1.

<sup>4</sup> Claesson, *Arkiv. Kemi, Min. Geol. A*, 1946, **24**, No. 7.

<sup>5</sup> Dole, *J. Amer. Chem. Soc.*, 1946, **42**, 769.

ultracentrifugation where their importance has been particularly pointed out by Ogston and Johnston.<sup>6</sup>

To make this clear we may consider a cell without adsorbent where two substances with the mobilities  $s$  (cm./sec.) and concentrations  $c$  indicated in Fig. 2 have been partly separated. If we consider two plane surfaces  $P_1$  and  $P_2$  in the position indicated in the figure the amount of solute 1 entering per second through  $P_1$  is  $s_{12}c_{12}$  and this amount is used up in moving the lower boundary the distance  $s_{11}$  and the upper boundary  $s_{22}$ . We consequently get

$$s_{12}c_{12} = s_{11}c_{11} + s_{22}(c_{12} - c_{11}), \quad . \quad . \quad . \quad (8)$$

$$\text{or} \quad c_{12} = c_{11} \frac{\left(1 - \frac{s_{11}}{s_{22}}\right)}{\left(1 - \frac{s_{12}}{s_{22}}\right)}. \quad . \quad . \quad . \quad (9)$$

It is therefore evident that as soon as the mobility is dependent on the concentration ( $s_{11} \neq s_{12}$ ) there will be a sudden change in the concentration at the boundary. The condensed formula (9) which is valid for all types of experiments will again take the form (6) if we include the substance taken up by the adsorbent and remember that the mobilities are inversely proportional to the retention volumes.

In fact, as eqn. (9) only contains ratios of mobilities it will be sufficient to assume that ratios of mobilities are independent of the total concentration in order to get simple results. That is true both for electrophoresis and ultracentrifugation.

It can be demonstrated<sup>4</sup> in the same way that eqn. (7) will be unchanged even if more complicated isotherms than Langmuir's are used as long as they have the same property as that isotherm, namely, that the ratio of the amounts adsorbed from a mixture is independent of the total concentration. That means that the isotherms for the different solutes can be of the form

$$a_i = k_i r(c_1 c_2 \dots c_n), \quad . \quad . \quad . \quad (10)$$

where  $r$  is a quite arbitrary function as long as it is the same for all the solutes.

When the theory for frontal analysis has been developed the corresponding simple theory for displacement development is almost self-evident. In displacement development introduced by Tiselius<sup>7</sup> in 1943 the substances to be separated are adsorbed at the top of the column. A solution of a substance with stronger adsorption than that of the unknown mixture is then forced through the column. This "developer" ( $d$ ) will then displace the different substances (1, 2) which also will displace each other. A diagram where the concentration of the effluent is plotted against the volume will therefore look as in Fig. 3, and the different components (1 and 2) will appear pure in the effluent. When a stationary state has been reached it is clear that all the boundaries will move down the column with the same rate as the front of the developer. As all the zones only contain one solute we

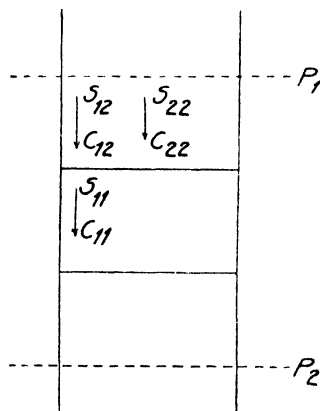


FIG. 2.

<sup>6</sup> Ogston and Johnston, *Trans. Faraday Soc.*, 1946, **42**, 769.

<sup>7</sup> Tiselius, *Arkiv. Kemi, Min. Geol. A*, 1943, **16**, No. 18.

can apply eqn. (I) and as the specific retention volume then can be regarded as a measure of the rate we get

$$\frac{f_1(c_1)}{c_1} = \frac{f_2(c_2)}{c_2} = \dots = \frac{f_d(c_d)}{c_d}, \quad (11)$$

where  $f_d(c_d)$  is the adsorption isotherm for the developer. Consequently if the concentration of the developer is kept fixed in all experiments a particular solute  $i$  always has to be of the same concentration  $c_i$  in order to fulfil eqn. (11). The height of a step is therefore independent of the amount of substance present and is a constant which can be used for identification of a substance. As the area of a step is proportional to the

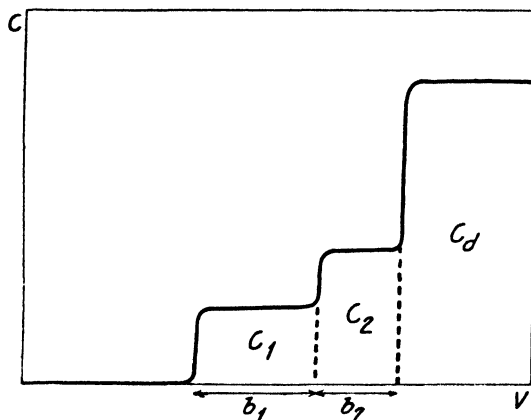


FIG. 3.

amount of substance present and its height is constant it is obvious that the length of a step ( $b$ ) is proportional to the amount of substance. We therefore get a qualitative analysis by measuring the heights of the steps and a quantitative by measuring their lengths.

Displacement development is therefore a very elegant way of carrying out chromatographic separations as it can be used for preparative, qualitative and quantitative purposes at the same time. It has, however, the drawback, common to all methods using reversible adsorption, that it may be difficult to find a developer which will displace the unknown mixture quantitatively.

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## THE CONSERVATION EQUATION OF CHROMATOGRAPHY

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The conservation equation of chromatography thus far used does not account for the volume occupied by the adsorbed phase. When the equation of conservation of matter is applied to the process of adsorptive percolation and this volume is accounted for, a general equation is obtained which may be more correct. This is shown by applying this equation to the equilibrium theory. Several effects occurring in chromatography which cannot be explained by the de Vault equation may now be interpreted, at least qualitatively. Moreover, the equation obtained reveals the possibility of a new method of adsorptive percolation.

In the development of the mathematical description of the process of adsorptive percolation two different methods of approach may be distinguished. One may start with the assumption of instantaneous equilibrium between solution and adsorbent (equilibrium theory) or one may dismiss the idea of complete equilibrium and treat the problem as a kinetic one (kinetic theory). However, in both cases the law of conservation of matter supplies the initial equation, which in both cases must be fundamentally the same. The equations obtained thus far give a ready explanation of the phenomena occurring during development. But when applying these equations in explanation of other processes of adsorptive percolation like the continuous introduction method (frontal analysis) or the elution method (displacement method), difficulties are encountered.

Moreover, the occurrence of so-called adsorption azeotropes,<sup>1</sup> especially with closely related compounds, needs further interpretation. Some experience gained with the adsorptive percolation of hydrocarbons suggested that the failures mentioned might be caused by the use of an approximate conservation equation.

### Symbols

The following symbols are used in this paper :—

- $a$ , interstitial volume of an adsorption column per unit of length (ml./cm.).
- $b$ , index referring to a sharp boundary.
- $C$ , concentration of solute expressed as units of weight of solute per unit of volume of solution (g./ml.).
- $C_A, C_B$ , concentrations of substance A and substance B, respectively.
- $d$ , density (g./ml.).
- $f_n(C_N)$ , adsorption isotherm of component  $N$ . It also represents the weight of solute adsorbed per unit of weight of adsorbent at the concentration  $C_N$  (g./g.).
- $f'_n(C_N)$ , first derivative of the adsorption isotherm ( $df_n(C_N)/dC_N$ ).
- $m$ , weight of adsorbent contained in the column per unit of length (g./cm.).
- $q$ , weight of solute adsorbed per unit of length of a column (g./cm.).
- $f(q)$ , volume occupied by the adsorbed phase (solute + solvent) per unit of length if a quantity  $q$  of solute is adsorbed (ml./cm.).
- $S$ , solvent.
- $V$ , volume of liquid forced into the column (ml.).
- $v$ , interstitial volume of dry adsorbent present in the column per unit of weight (ml./g.).
- $x$ , distance from the top of the column (cm.).
- $0, 1$  (when combined with  $C$ ) indices indicating zero and unit concentration respectively.
- $1, 2, 3$  (when combined with  $f$ ) indices indicating that the functions  $f_1, f_2$  and  $f_3$  are different functions.

**1. The Conservation Equations used thus far.**—The first conservation equation was given by Wilson <sup>2</sup> :

$$(\partial C / \partial x)_V + (\partial q / \partial V)_x = 0, \quad . \quad . \quad (1)$$

where  $C$  is the concentration of the solute,  $x$  the distance from the top of the column,  $q$  the weight of solute adsorbed per unit of length and  $V$  the volume of liquid poured into the adsorption column. This equation has been corrected by de Vault <sup>3</sup> for the interstitial volume  $a$  per unit of length of the adsorption column.

De Vault arrives at the equation :

$$(\partial C / \partial x)_V + a(\partial C / \partial V)_x + (\partial q / \partial V)_x = 0. \quad . \quad . \quad (2)$$

<sup>1</sup> Hirschler and Amon, *Ind. Eng. Chem.*, 1947, **39**, 1565.

<sup>2</sup> Wilson, *J. Amer. Chem. Soc.*, 1940, **62**, 1583.

<sup>3</sup> De Vault, *J. Amer. Chem. Soc.*, 1943, **65**, 532. \*

The same equation has been used implicitly or explicitly by other authors<sup>4</sup> whether they adopted the equilibrium or the kinetic standpoint, for the equilibrium or the kinetic conditions are introduced subsequently when the relation between  $C$  and  $q$  is inserted. Eqn. (2) is based on the assumption that the interstitial volume  $a$  is constant. This is the point where introduction of a correction might be useful. The adsorbed phase occupies a certain fraction of the original interstitial volume thus causing the liquid to flow at a greater linear rate than when the adsorbed phase is not present.

As the quantity of material adsorbed is a function of the concentration the *free* interstitial volume, that is, the volume available to the *flowing* liquid, must be a function of the concentration.

**2. Derivation of the Corrected Conservation Equation.**—To introduce the correction for the volume of the adsorbed phase the conservation equation will be derived once more for a solution containing a single solute. The following assumptions, which do not differ from those usually applied, have been made. Within an adsorption band the concentration is a continuous function of  $x$ . Within a cross-section of infinitesimal length  $dx$ ,  $(\partial C/\partial x)_V$  is considered constant. The same applies to  $(\partial q/\partial V)_x$  when an infinitesimal volume of liquid  $dV$  is introduced into the column.

When an infinitesimal volume  $dV$  is introduced into the column an equal volume has to pass through a cross-section at  $x$ , where  $x$  is a point within the adsorption band. As the concentration of the solution entering the cross-section at  $x$  and the concentration of the solution leaving the cross-section at  $(x + dx)$  differ by

$$(\partial C/\partial x)_V dx,$$

the amount of solute present in this section of the column is increased by

$$(\partial C/\partial x)_V dx.dV.$$

This amount is distributed between the liquid phase and the adsorbed phase present in the section. The volume of the liquid phase present in the section is equal to

$$(a - f(q))dx,$$

where  $f(q)$  means the volume of the adsorbed phase per unit of length within the section considered. Thus the amount of solute present in the liquid phase is equal to

$$(a - f(q))Cdx.$$

The amount of solute present in the adsorbed phase is equal to  $qdx$ , where  $q_n = mf_n(C_N)$ . Thus the total amount of solute present in the section is equal to

$$\{(a - f(q))C + q\}dx.$$

If an infinitesimal volume  $dV$  is forced through this section, the increase of solute may therefore also be represented by

$$\frac{\partial}{\partial V} [(a - f(q))C + q]_x dx.dV.$$

According to the law of conservation of matter the amount of solute introduced by the volume  $dV$  should be equal to the increase of the amount of solute present in the section. Thus

$$\left(\frac{\partial C}{\partial x}\right)_V dx.dV + \frac{\partial}{\partial V} [(a - f(q))C + q]_x dx.dV = 0, \quad (3)$$

or

$$(\partial C/\partial x)_V + \{a - f(q)\}(\partial C/\partial V)_x - \{\partial f(q)/\partial V\}_x C + (\partial q/\partial V)_x = 0. \quad (3)$$

<sup>4</sup> Martin and Synge, *Biochem. J.*, 1941, **35**, 1941. Weiss, *J. Chem. Soc.*, 1943, **145**, 297. Thomas, *Ann. N.Y. Acad. Sci.*, 1948, **49**, 161.

This is the corrected conservation equation of chromatography in its general form for a solution containing a single solute.

As to  $f(q)$ , it should be borne in mind that it is not correct to put

$$f(q) = q/d,$$

where  $d$  means the density of the adsorbed solute. For besides the solute the solvent is adsorbed too. As to this point it might be useful to stress that adsorption isotherms of pure gases and so-called isotherms of solutions containing a single solute usually have a different meaning. The adsorption isotherm of a pure gas shows the relation between the concentration (pressure) of the gas and the weight adsorbed.

However, the so-called adsorption isotherm of a solute shows the relation between the concentration and the weight of solute adsorbed *preferentially*. This relation might be better called a preferential adsorption isotherm. To avoid confusion it may be stated here that in this paper the adsorption isotherm of a solute means the relation between the *total* amount of solute adsorbed per unit of weight of adsorbent and the concentration of the solution with which it is in equilibrium.

**3. Application of Eqn. (3) to the Equilibrium Theory.**—Attempts will now be made to derive a fundamental equation of chromatography on the basis of the equilibrium theory for a liquid mixture of two substances A and B. Which of the two is considered as solvent does not matter. For the sake of convenience it is assumed that A and B have equal densities and no volume change occurs on adsorption or mixing. If the densities of A and B are represented by  $d$  and the concentration is expressed as g./ml., we may put

$$C_A + C_B = d.$$

Suppose the adsorption column considered contains  $m$  g. adsorbent per unit of length. The adsorption isotherm of A when diluted by B is represented by  $f_1(C_A)$ . At equilibrium,

$$q = mf_1(C_A)$$

(for non-equilibrium another relation between  $q$  and  $C$  should be introduced but this will not be discussed). If  $f_2(C_B)$  represents the adsorption isotherm of B when diluted by A we may put

$$f(q) = \{f_1(C_A) + f_2(C_B)\}m/d.$$

If  $v$  is the interstitial volume of the dry adsorbent per unit of weight,

$$a = mv.$$

Substitution in eqn. (3) gives

$$(\partial C_A / \partial x)_V + m[\{v - f_1(C_A)/d - f_2(C_B)/d\}(\partial C_A / \partial V)_x - \{(\partial f_1(C_A) / \partial V)_x + (\partial f_2(C_B) / \partial V)_x\}C_A/d + (\partial f_1(C_A) / \partial V)_x] = 0.$$

On further substitution, viz.,

$$(\partial C_A / \partial x)_V = -(\partial V / \partial x)_{C_A} (\partial C_A / \partial V)_x,$$

$$(\partial f_1(C_A) / \partial V)_x = (\partial f_1(C_A) / \partial C_A) (\partial C_A / \partial V)_x = f'_1(C_A) (\partial C_A / \partial V)_x$$

$$\text{and } (\partial f_2(C_B) / \partial V)_x = (\partial f_2(C_B) / \partial C_B) (\partial C_B / \partial V)_x = -f'_2(C_B) (\partial C_A / \partial V)_x,$$

the following equation is obtained :

$$-(\partial V / \partial x)_{C_A} (\partial C_A / \partial V)_x + m[v - f_1(C_A)/d - f_2(C_B)/d - \{f'_1(C_A) - f'_2(C_B)\}C_A/d + f'_1(C_A)](\partial C_A / \partial V)_x = 0,$$

or

$$-(\partial V / \partial x)_{C_A} + [vd - f_1(C_A) - f_2(C_B) + C_B f'_1(C_A) + C_A f'_2(C_B)]m/d = 0.$$

Thus

$$(\partial x / \partial V)_{C_A} = \frac{d}{m[vd - f_1(C_A) - f_2(C_B) + C_B f'_1(C_A) + C_A f'_2(C_B)]} \quad (4)$$

De Vault,<sup>3</sup> whose paper has been very useful to the author, assumed the interstitial volume to be constant and obtained

$$(\partial x / \partial V)_{C_A} = \frac{I}{m[v + f'_1(C_A)]} \quad (4a)$$

According to the initial assumptions, eqn. (4) holds good only within the adsorption band. In the same way, as de Vault did, it can be shown that at a boundary of an adsorption band eqn. (4) may lead to physical impossibilities, thus indicating the occurrence of a discontinuity (sharp boundary).

If  $V$  is increased at a constant rate,  $(\partial x / \partial V)_{C_A}$  may be called the rate of transport of the concentration  $C_A$ . If  $(\partial x / \partial V)_{C_A}$  increases with increasing concentrations, thus if

$$\partial\{(\partial x / \partial V)_{C_A}\} / \partial C_A \text{ is positive,}$$

it means that the highest concentration of A possible will move at the greatest rate. Consequently it will overtake all lower concentrations of A which might be in front of it. Thus a sharp front boundary will be finally formed and the lowest concentration possible will be trailing behind forming a diffuse boundary. If, however,

$$\partial\{(\partial x / \partial V)_{C_A}\} / \partial C_A \text{ is negative,}$$

the inverse will be obtained.

A further calculation, which will not be extended here, shows that if adsorption occurs according to the Langmuir theory of adsorption a leading front boundary of A will be formed if

$$f_1(C_A)/C_A > f_2(C_B)/C_B$$

holds good at all concentrations. This simple condition only means that the concentration of A in the liquid phase decreases if adsorbent is added to any mixture of A and B. If the adsorption isotherms of A and B are of the Freundlich type and

$$f_1(C_A)/C_A > f_2(C_B)/C_B,$$

a leading front boundary will also be formed, at least at low or high concentrations. At medium concentrations no general conclusion can be drawn. Thus it may be assumed, if

$$f_1(C_A)/C_A > f_2(C_B)/C_B,$$

a sharp front boundary of A will generally be formed.

As has already been stated, eqn. (4) only holds good within an adsorption band. When a sharp boundary occurs, whether in front or in the rear, another conservation equation has to be applied to the boundary. For either in front or at the rear of such a boundary, no trace of the substance considered should be present.

Therefore the conservation equation of a sharp boundary is

$$CdV = \{(a - f(q))C + q\}dx, \quad (5)$$

where  $C$  applies to the concentration in the boundary. If eqn. (5) is applied to the mixture of A and B and equilibrium is instantaneously established the following equation holds good for a sharp boundary:

$$\left(\frac{\partial x}{\partial V}\right)_{C_{A_b}} = \frac{d}{m \left\{ vd + C_{B_b} \left( \frac{f_1(C_{A_b})}{C_{A_b}} - \frac{f_2(C_{B_b})}{C_{B_b}} \right) \right\}}, \quad (6)$$

where the subscript  $b$  indicates that the concentration refers to that in the sharp boundary.

For the diffuse boundary (zero concentration) the following equation is obtained :

$$\left(\frac{\partial x}{\partial V}\right)_{C_{A_0}} = \frac{I}{m\{v + f'_1(C_{A_0}) - f_2(C_{B_1})/d\}}, \quad (7)$$

where the subscripts 0 and 1 indicate zero and unit concentration  $d$  respectively. Applying de Vault's theory,

$$\left(\frac{\partial x}{\partial V}\right)_{C_{A_b}} = \frac{I}{m(v + f_1(C_{A_b})/C_{A_b})} \quad (6a)$$

and

$$\left(\frac{\partial x}{\partial V}\right)_{C_{A_0}} = \frac{I}{m(v + f'_1(C_{A_0}))} \quad (7a)$$

are obtained.

**4. Discussion.**—Eqn. (4) represents the rate of transport of a certain concentration of A within the adsorption band. But the corresponding concentration of B should attain the same value. Thus

$$(\partial x/\partial V)_{C_A} = (\partial x/\partial V)_{C_B},$$

if  $C_B = d - C_A$ . Eqn. (4) meets this condition whereas eqn. (4a) does not, unless

$$f'_1(C_A) = f'_2(C_B),$$

which as a rule cannot be true.

As has already been shown, a sharp leading boundary of A will be formed if

$$f_1(C_A)/C_A > f_2(C_B)/C_B.$$

Consequently

$$(\partial x/\partial V)_{C_{A_b}} \leq \frac{I}{mvd} \text{ (eqn. (6)).}$$

This means that the rate of transport of any boundary is always smaller than the rate of transport of the liquid front, which of course is equal to  $I/mvd$ . Both rates become equal if  $C_B = 0$ , i.e.,  $C_A = d$ . If, however,

$$f_1(C_A)/C_A < f_2(C_B)/C_B,$$

the front boundary will be diffuse and a sharp boundary will be present at the rear. In this case the rate of transport of the boundaries is always greater than the rate of transport of the liquid front, unless  $C_A = d$ . Thus it may be stated if

$$f_1(C_A)/C_A > f_2(C_B)/C_B,$$

substance A will always lose on the liquid front, whereas B has a tendency to overtake the liquid front until its concentration  $d$  has become equal to unity. This may serve as a suitable interpretation of the phenomena which obtain when a solution containing a single solute is introduced into the dry column (continuous introduction method). If a mixture of A and B is introduced into a dry column, A will move at a slower rate than the liquid front.

Therefore pure B will appear in front of the adsorption band and its quantity will increase if introduction is continued. Since at the top of the column the concentration of A is equal to the initial concentration and this is the highest concentration of A possible under these conditions, it will overtake all lower concentrations of A which might be in front of it. Thus within the adsorption band the concentration of A is equal to the initial concentration and the adsorption band will move at a constant rate.

Elution (displacement) may now be explained too. Suppose two substances A and B are present in an adsorption column and a new solvent S is added at the top of the column. Further

$$f_3(C_S)/C_S > f_1(C_A)/C_A > f_2(C_B)/C_B.$$



According to the above deductions, any A or B which happens to become diluted by S will move at a greater rate than the liquid front, whereas the rate of S should be smaller than (or, when undiluted, equal to) the rate of the liquid front. Therefore S will remain undiluted and act like a piston forcing A and B in front of it. The same applies to A as compared to B. Therefore the final result of elution will be three adjacent adsorption bands containing S, A and B at unit concentrations. As to the developer method, little further explanation seems to be necessary. If more than one solute is present and separation of the solutes has been obtained already, the rates of transport of the different solutes are established by eqn. (6) and (7) and thus the distance between the adsorption bands will usually increase on further development and at the same time the bands will be broadened.

The mere process of separation of a mixture of solutes is more complicated because of mutual alteration of the adsorption isotherms. Nevertheless, it may be accepted that the separation itself occurs qualitatively along the same lines. As a rule when the developer method is applied a sharp front boundary occurs. Experimentally asymmetric adsorption bands are usually found, the highest concentration being near the front.

Eqn. (6) and (7) reveal another possibility which may not as yet have been recognized. Suppose

$$f_1(C_A)/C_A < f_2(C_B)/C_B.$$

An adsorption band of A when present will move at a greater rate than the liquid front. But if the whole column has previously been wetted with B, the substance A cannot reach the liquid front before leaving the column. If a mixture of A and B is introduced into a column previously wetted with B, the lowest concentration of A will move at the greatest rate. Thus an adsorption band with a diffuse front and a sharp rear boundary will be formed immediately. This band is transported through the column if there-upon pure B is added to the column at the top. The band is broadened during this procedure but its rate is conditioned by eqn. (6) and (7). If still another solute, say, D, is present which is also adsorbed less strongly than B it will behave in the same way, but it may be that both limits of the rate of transport of the adsorption band of D are smaller than those of B. Thus this method, which is supposed to be called development with an eluent, may cause separation.

According to former conceptions this method would be impossible because B, the eluent, should not allow A or D to become adsorbed. However, a very simple experiment showed the present conception to be correct. A mixture of cetane and cetene could be partly separated by silica gel previously wetted with benzene, using benzene as developing liquid. It is well known that benzene may act as an eluent (displacer) for cetane and cetene when adsorbed on silica gel. The advantage of this method is that the total volume of "developer" necessary to collect all the solutes at the bottom of the column is smaller than the interstitial volume of the column. This means a saving of time. However, the separating efficiency of this method may be, in many cases, low.

The occurrence of rates of transport greater than the rate of the liquid front is also demonstrated in an earlier paper,<sup>5</sup> though not mentioned explicitly. Rates of transport up to about 1.3 times the rate of the liquid front have been found with chloroform and dodecylbenzene.

A few words may be added as to the occurrence of so-called adsorption azeotropes as described by Hirschler and Amon.<sup>1</sup> When an adsorption azeotrope occurs

$$f_1(C_A)/C_A = f_2(C_B)/C_B$$

<sup>5</sup> Smit, *Anal. chim. Acta*, 1948, **2**, 671.

changes sign at a certain concentration of, say, A. At concentrations lower than the azeotropic concentration the sign may be positive, and when the mixture is introduced into the column pure B appears in front of the adsorption band containing A. Beyond the azeotropic concentration pure A will appear in front, and B is contained within the adsorption band.

At the azeotropic concentration

$$f_1(C_A)/C_A - f_2(C_B)/C_B = 0.$$

The rate of transport of A and B both become equal to the rate of the liquid front and no separation occurs. So the conclusion derived from eqn. (6) and (7) agrees with experiment. At the same time it is clear that the occurrence of adsorption azeotropes is limited to substances having comparable adsorption affinities.

No attempt has been made to solve the differential equations, nor to develop formulæ for more than two substances. As de Vault already stated, this becomes very complicated. Moreover, as real adsorption isotherms are used in our equation, the quantitative solution is of no use as the real adsorption isotherms are not available. The main purpose of this paper has been to arrive at a rather simple formula which permits a qualitative explanation of the different methods of percolation, but on the other hand it might show that the quantitative deductions made thus far have to be handled with care.

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*Koninklijke/Shell-Laboratorium,  
Amsterdam.*

## GENERAL DISCUSSION \*

**Dr. B. Davison** (*A.E.R.E., Harwell*) (*communicated*): I want to point out that for the case of Langmuir isotherms the method described by Dr. Glueckauf in his paper can be easily generalized to any number of solutes simultaneously present in solution. For simplicity I shall speak of three solutes only.

The basic equations are

$$\frac{df_1}{dc_1} = \frac{df_2}{dc_2} = \frac{df_3}{dc_3}, \quad . \quad . \quad . \quad . \quad (1)$$

in which

$$f_j = \frac{a_j c_j}{1 + b_1 c_1 + b_2 c_2 + b_3 c_3} \quad . \quad . \quad . \quad . \quad (2)$$

Putting

$$p_j = b_j c_j; \quad P = p_1 + p_2 + p_3 \quad . \quad . \quad . \quad . \quad (3)$$

and re-writing (1) in terms of  $p_j$ 's and  $P$ , differentiating again using  $P$  as independent variable, and comparing with (3) we get †

$$p_1 p_2 p_3 \left[ \frac{1}{a_1 p_1} \left( \frac{dp_1}{dP} \right)^2 + \frac{1}{a_2 p_2} \left( \frac{dp_2}{dP} \right)^2 + \frac{1}{a_3 p_3} \left( \frac{dp_3}{dP} \right)^2 \right] = 0, \quad . \quad (4)$$

\* On four preceding papers.

† This derivation presupposes that none of  $c_j$ 's is constant and  $P$  is not constant either. But assuming all  $a_j$ 's to be different, one can easily show that  $P = \text{const.}$  is irreconcilable with eqn. (1) and can occur only in the regions where all concentrations are constant; consequently the eqn. (1) is not applicable. If one of the concentrations is constant, one can similarly show that either  $\bar{P}$  is constant, or the concentration in question is zero and we have one solute less.



boundary, while for a sharp boundary it decreases. This generalizes Glueckauf's Rule 2. The conclusions are illustrated on the diagrams.

Fig. 1a (continuous line) gives the  $S$  diagram for the case when one set of three concentrations  $c_1^\circ, c_2^\circ, c_3^\circ$  is developed by another set of three concentrations  $\bar{c}_1, \bar{c}_2, \bar{c}_3$  of the same solutes, and these  $c_j^\circ, \bar{c}_j$  are such that all  $S_j^\circ > \bar{S}_j$  (all three

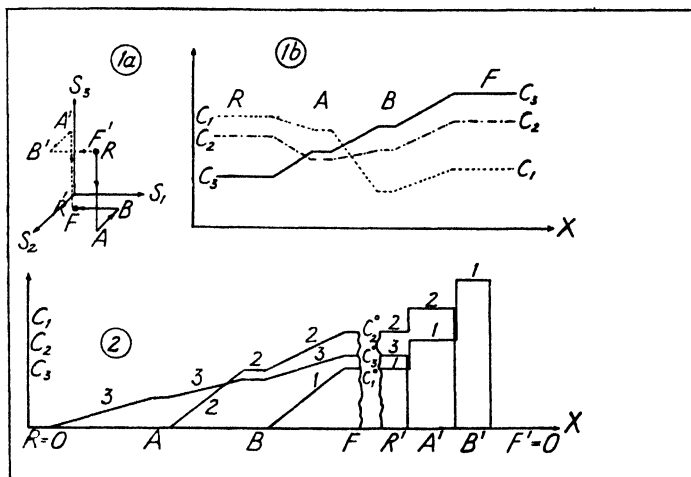


FIG. 1a, b and 2.

boundaries are diffuse). Fig. 1b gives schematically the corresponding concentration diagram. The case of three diffuse boundaries will arise, for instance, if a set of three concentrations is developed by clear solvent. In this latter case the concentration diagram reduces to that represented in Fig. 2 (rear part). The dotted line in Fig. 1a gives the  $S$  diagram for the case when, on the contrary,  $\bar{c}_1, \bar{c}_2, \bar{c}_3$  are developed by  $c_1^\circ, c_2^\circ, c_3^\circ$ . The corresponding concentrations diagram for  $\bar{c}_1 = \bar{c}_2 = \bar{c}_3 = 0$  is given in the front part of Fig. 2.

**Dr. F. G. Angell** (*Stockton-upon-Tees*) said: From the standpoint of a practical chromatographer it is worth while exploring, or if necessary experimentally determining, the adsorption isotherms of two substances on a particular adsorbent before proceeding with any chromatographic separation. According to Dr. Glueckauf's paper, conditions for sharp boundaries in adsorption chromatography have been uniquely defined and provided that equilibrium is established under experimental conditions it would appear that much time and labour could be spared and more chances of success accrue by such a procedure than by the more customary empirical method of trial and error with various adsorbents.

**Dr. E. Glueckauf** (*A.E.R.E., Harwell*) said: Dr. Smit mentions the need for further knowledge about chromatography in the case of adsorption azeotropes. Actually we can predict fairly well what will happen in such cases provided that the binary isotherms are at least approximately known. Let us assume, as an example, that these isotherms are of the exponential type:

$$a_1 c_1 = q_1 (q_1 + q_2)^{(n_1-1)} \text{ etc.}^1$$

where at low concentrations solute 2 is the more strongly adsorbed, and where  $n_2 > n_1 > 1$ .

Such a system gives adsorption azeotropes whenever the total adsorption density

$$(q_1 + q_2) = \frac{a_2}{a_1} \exp \left( \frac{1}{n_2 - n_1} \right).$$

<sup>1</sup> See Glueckauf, *J. Chem. Soc.*, 1947, 1302.

The  $q_1$ - $q_2$  characteristics (see preceding paper by Glueckauf) of co-existing concentrations have approximately the form shown in Fig. 1 (only one positive characteristic being shown). The essential difference from the normal type of characteristic is that the positive characteristic is convex against the point  $q_1 = 0, q_2 = 0$ .

Applying the rules given in the paper earlier in this Discussion we can see that nothing abnormal will happen during solvent development, if the original starting point  $q_1^0, q_2^0$  lies below the azeotropic line DZE. If lying above this line, the following complications will occur: at first, solute 2 will separate both in rear and front, with enrichment of solute 1 in the centre of the mixed band. Due to the spreading of the band during solvent development, the maximum concentration of the mixed band moves along the line BZA. After Z has been passed the formerly sharp front of the mixed band becomes a diffuse one; solute 1 enters the frontal band of solute 2, and eventually overtakes it. After a somewhat complex state of affairs (which can in detail be deduced from the diagram of characteristics), we arrive at the shape of the normal band distribution with a frontal band of pure solute 1. This procedure wastes a good deal of time and adsorbent in the process; which could be saved by working from the start at lower adsorption densities, i.e., with more dilute

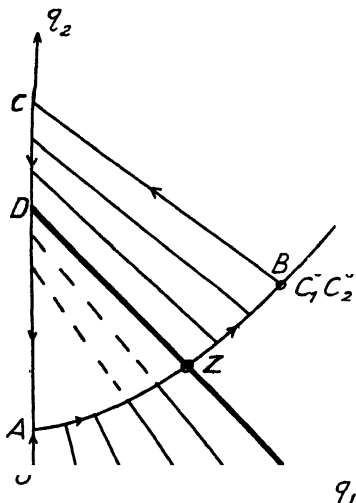


FIG. 1.

solutes. One can compare this process with the distillation of binary azeotropes, after the addition of a third inert solvent.

**Dr. W. M. Smit** (*Amsterdam*) (*communicated*): In stating that there is a need of further knowledge on the behaviour of adsorption azeotropes it has not been my intention to say that it is not yet possible to give a qualitative prediction of what will happen in an adsorption column if an adsorption azeotrope occurs.

When "more strongly adsorbed" stands for "moving at a lower rate" a qualitative prediction of the phenomena which will occur in the case of adsorption azeotropes is even possible without using any formula. By simple reasoning we may come to the same conclusions as arrived at by Dr. Glueckauf.

However, the adsorption azeotropes have been mentioned with a different purpose, viz., to demonstrate the need of correcting the conservation equation.

If a solution of azeotropic concentration is poured into an adsorption column no separation occurs and the adsorption front will move at the same rate as the liquid front. The equations for the rate of transport of the front of an adsorption band derived from the conservation equations of Wilson or of de Vault (see paper on the conservation equation) lead to conclusions which do not tally with this simple fact.

De Vault's equation only gives the right value for the rate of transport of the adsorption front if the amount adsorbed at the azeotropic concentration is zero, which cannot be true. The same obtains for the rate of transport of the adsorption front of a pure solvent containing no solutes.

Another point is that Dr. Glueckauf refers to ternary systems (a solvent with two solutes) whereas the literature quoted by me deals with binary systems (a solvent with one solute).

Finally, an interesting feature is that in the case of a binary system de Vault's conservation equation will lead to the same results as the corrected equation if  $f(C_A)$  in de Vault's equation is replaced by another function  $F(C_A)$  where  $F(C_A)$  stands for the amount of substance A adsorbed *preferentially*. But this

implies that  $F(C_A)$  cannot be represented by an adsorption isotherm of the Langmuir type, the Freundlich type or the exponential type as given by Dr. Glueckauf. For  $F(C_A)$  should at least meet the following condition:  $F(C_A) = 0$ , for  $C_A = 0$  and  $C_A = 1$ . It may be a point for further investigation to determine how the equations for solutions containing more than one solute are affected by using the corrected conservation equation.

**Dr. B. Davison** (*A.E.R.E., Harwell*) said: The treatment of the problem given by Prof. A. C. Offord and Dr. J. Weiss in their paper is, in many respects, inadequate. In particular, when confronted with a situation when at the first sight there are several alternatives, they invariably choose the alternative which a more detailed analysis shows to be unacceptable. This can be most clearly seen if we re-examine the examples considered in their paper.

Let me first draw attention to formula (23). It will be noticed that the value of  $c_1'$  given by this formula depends only upon the form of  $q_1$  and  $q_2$  as functions of  $c_1$  and  $c_2$ , and not upon the concentrations which were initially present.

For instance for the Langmuir isotherms

$$q_1 = \frac{a_1 c_1}{1 + b_1 c_1 + b_2 c_2} ; \quad q_2 = \frac{a_2 c_2}{1 + b_1 c_1 + b_2 c_2},$$

the formula (23) leads to

$$c_1' = \frac{1}{b_1} \frac{a_1 - a_2}{a_2}.$$

It is rather difficult to visualize how this is possible, in particular if the initial concentration  $c_1^0$  of the first solute is very much smaller than the value of  $c_1'$  given by (23). Indeed if  $a_2 \ll a_1$  i.e., if solute 2 is little adsorbed, impossibly high values of  $c_1'$  would be postulated by eqn. (23). Eqn. (23) also leads to the interesting value (26.2) for the content of solute 1 in the pure rear band, which can exceed the total amount of solute 1 introduced into the column.

This suggests that we should re-examine the derivation of (23). The formula (23) was derived from the assumption that (7) should hold for all values of  $v/x$ . But (7) was derived as the condition for the eqn. (6) to have other solutions than  $dc_1/dy = dc_2/dy = 0$ . Thus all we can say is that in the vicinity of any particular value of  $v/x$  either (7) should hold, or both concentrations should be constant.

Let us see what will happen if we introduce a constant concentration band between the regions of  $c_2 > 0$  and  $c_2 = 0$ ,  $c_1$  variable. Then at one boundary of this region

$$v/x = \left( \frac{\partial q_2}{\partial c_2} \right)_{c_2=0},$$

at the other

$$v/x = \left( \frac{\partial q_1}{\partial c_1} \right)_{c_1=0},$$

and the impossible eqn. (23) (or, at any rate, the equation with which one finds a great difficulty to reconcile oneself) disappears. This can be taken as a proof that, at least for Langmuir isotherms, there will necessarily be a constant concentration band separating the regions  $c_2 > 0$  and  $c_2 = 0$ ,  $c_1$  variable. In the light of these remarks formulæ (26) must be modified (and perhaps also some other formulæ).

I also want to draw attention to formulæ (30) which postulates two discontinuities separated by a mixed band. For the sake of clarity I shall apply them to the case of Langmuir isotherms, where their inadequacy becomes very obvious. According to the assumption that solute 1 is more strongly adsorbed,  $a_1 > a_2$ .

Now if the postulated mixed band is to be formed spontaneously between two discontinuities, the boundary between the mixed band and the original solute should move faster than the boundary between the mixed band and the pure solution of the developing solute. In the notation of formula (31) it follows that

$$x_2 > x_1, \quad v/x_2 < v/x_1$$

and, according to (31),

$$\frac{q_1(c_1', c_2')}{c_1'} < \frac{q_2(c_1', c_2')}{c_2'}.$$

For Langmuir isotherms, this becomes

$$\frac{a_1}{1 + b_1 c_1' + b_2 c_2'} < \frac{a_2}{1 + b_1 c_1' + b_2 c_2'},$$

i.e.,  $a_1 < a_2$

contrary to the assumption that solute 1 is more strongly adsorbed.

To resolve this contradiction let us re-examine the derivation of (30). Formulæ (30) is an application of formula (2.3)

$$\frac{q_1(\text{II}) - q_1(\text{I})}{c_1(\text{II}) - c_1(\text{I})} = \frac{q_2(\text{II}) - q_2(\text{I})}{c_2(\text{II}) - c_2(\text{I})} \quad (a)$$

derived from the conservation of material of both solutes. It is essential for the derivation of this formula that both solutes be present at the boundary. If, for instance, the solute 1 is not present on either side of the boundary, then the eqn. (2.1) disappears and we can no longer use equation (a).

If it is known beforehand that on one side of the boundary both solutes are present, then, of course, the formula (2.3) is necessarily satisfied. But if on one side of the boundary only one solute, say, solute 2, is present, and we want to determine the concentrations on the other side of the boundary, then there are two possibilities.

Either these concentrations will be determined by eqn. (a) or only the solute 2 is present and  $c_1' = 0$ . In the example under consideration we have thus three possibilities for the concentrations between the two discontinuities, namely

$$(a) \quad c_1' = 0 \qquad (b) \quad c_2' = 0 \qquad (c) \quad c_1' \text{ and } c_2'$$

determined by the eqn. (30).

We have already seen that the possibility (c)—the one chosen by Offord and Weiss—leads, at least in the case of Langmuir isotherms, to contradiction.

The possibility (b), i.e., that of  $c_2' = 0$ , also leads, at least in the case of Langmuir isotherms, to the same contradiction ( $x_1 = x_2(1 + b_1 c_1)$  and hence  $x_1 > x_2$ ).

But the possibility (a), i.e., that of  $c_1' = 0$ , leads to  $x_2 = x_1(1 + b_2 c_2)$  and hence  $x_2 > x_1$  as it ought to be. Thus the possibility (a) is the only acceptable one.

I should also point out that if one eliminates from Offord and Weiss's paper all the unacceptable choices of alternatives, and uses the logically acceptable ones, one arrives at the same results as have been already published by Glueckauf.

**Dr. E. Glueckauf** (*A.E.R.E., Harwell*) said: I wish to call Prof. Offord's attention to eqn. (17) which is a simple quadratic for  $y$ . In view of eqn. (12),  $q_1$  and  $q_2$  are both functions of  $c_1$  and  $c_2$  only, and

$$(\partial q_1 / \partial c_1)_{c_2=0} \text{ and } (\partial q_2 / \partial c_2)_{c_1=0}$$

have thus values independent of  $y$ .

I ask Prof. Offord why he postulates (see eqn. 23) that the two roots of the quadratic (17) should be equal, when this is absolutely impossible. This answer is essential, because the rest of paragraph II is based on eqn. (23).

In order to avoid confusion in future research, I wish to remove any impression that there are two alternative theories or even two alternative ways of approach in this matter. Actually the fundamental approach is very similar as can be seen from the identity of a number of differential equations, and logical treatment of these equations would lead anybody to the same results, as were obtained in my researches during the last five years.

However, the meaning which these equations take on after integration, and the physical limits imposed by the fact that we are dealing with physical realities, have sometimes escaped the authors, and of several mathematically possible solutions they have repeatedly chosen the physically impossible ones. Thus we are confronted with concentration bands which, beginning with a zero-existence, have a negative growth-rate; with other bands which can contain more solute than has been put into the column.

In paragraph I, the treatment of a sharp boundary between two binary solutions (i.e., eqn. (2.3), (3), and Fig. 1) is inadequate and misleading. In paragraph II, all equations after (21) are erroneous, including Fig. 2. In paragraph III all conclusions after eqn. (29) are wrong, including Fig. 3. In paragraph IV all conclusions are wrong, including Fig. 4.

I must apologize to Prof. Offord and Dr. Weiss for stating this so bluntly, the reason being that the Fig. 2, 3, 4 look so extremely plausible to the chemist who uses chromatography as a technical tool. The disturbing effects of using a finite grain-size and of non-equilibrium phenomena, which have *not* been taken into account in their papers, result in exactly those phenomena which Offord and Weiss claim to have found for an "ideal" column by means of their wrong interpretations. These disturbing phenomena cause a smearing-out of the flat region, missing at  $X_1'$  in their Fig. 2, and they cause variable mixed boundaries under the conditions of Fig. 3 and 4. But *these* phenomena though shown in the Figures are *not* represented by the equations of Offord and Weiss.

**Prof. A. C. Offord** (*London*) (*partly contributed*): In spite of the remarks of Dr. Glueckauf I must emphasize that our approach to the problem is fundamentally different from his and any points of resemblance in the two theories are largely a matter of accident.

One of the main problems treated in both contributions is the development of a chromatogram consisting of a band of two substances by pure solvent. Dr. Glueckauf starts with the simultaneous partial differential equations

$$\frac{\partial c_i}{\partial x} + \frac{\partial f_i}{\partial v} = 0, \quad . \quad . \quad . \quad . \quad . \quad (1)$$

where  $i = 1, 2$  and  $f_1$  and  $f_2$  are functions of  $c_1$  and  $c_2$  only. These equations are subject to the initial conditions that  $c_1 = c_2 = 0$  when  $x = 0$ , and that  $c_1 = c_1^0$  and  $c_2 = c_2^0$  when  $v = 0$ . He then gives what is claimed to be a solution of these equations, which is broadly of the following form. The first quadrant is divided into five sectors by four straight lines (i) (ii) (iii) and (iv) issuing from the origin, and the functions  $c_1$  and  $c_2$  are given by various expressions, different for each sector. This solution has, however, no mathematical validity, for the partial derivatives of the functions  $c_1$  and  $c_2$  do not exist on the lines (i) (ii) (iii) and (iv). In fact, these differential equations have no solutions under the boundary conditions stated.

Our contribution was a continuation of the work of Weiss<sup>2</sup> on the problem for a single solute and it has much in common with this earlier investigation. The mathematical treatment for the single solute (see the Appendix<sup>2</sup>) hinges on the fact that we are not dealing with a sharp discontinuity at the top of the band, but with a continuous tailing-off. Indeed, an explicit form was assumed for this tail off (see eqn. (A 13)). The conclusion arrived at was, however, that, in the case of isotherms with pronounced curvature, the major term, giving the concentration in the rear part of the elute, was in fact independent of the initial shape assumed for the top of the band. Hence, in spite of the fact that in its final form the result appears to depend on the displacement relations only, it was only by making use of the notion of a diffused initial boundary (created by the presence of diffusion) that the problem could be given a tangible mathematical form.

Unfortunately the same treatment cannot be applied in the case of two solutes, but none the less similar considerations apply. The main point remains, that the boundary conditions  $c_1 = c_2 = 0$  when  $x = 0$ , and  $c_1 = c_1^0$ ,  $c_2 = c_2^0$ , when  $v = 0$ , have neither mathematical nor physical validity, and it is essential, not only for physical but also for mathematical reasons to treat this boundary as a diffused boundary which it most certainly is. If we fail to do this we do not arrive at an "ideal" problem, as asserted by Dr. Glueckauf, but at one which has no meaning.

It should have been explained that our treatment only applies when the isotherms have a pronounced curvature. The results we give are not true for



linear isotherms, where the phenomenon of diffusion plays a very much more marked role. Unlike Dr. Glueckauf, we can lay no claim to a definitive mathematical theory valid in all cases, and, in our communication, we considered only a few problems which we regarded as of particular interest. In the case of the special problem under consideration, we remarked that various other solutions were possible depending on the particular isotherms. This applies to the particular example selected by Dr. Davison, where the more strongly adsorbed substance is present only in weak concentration. Our solution does not apply in this case. If one substance is present only as a trace, then another mathematical treatment is possible and we hope to return to this problem later.

Dr. Glueckauf's main objection arises from our treatment of eqn. (7) in our paper<sup>3</sup> leading to eqn. (23), which is vital for all further consideration. This treatment is, however, necessary if the eqn. (1) are to have a solution (with continuous partial derivatives) valid over the part of the chromatogram where the faster moving solute first appears. Eqn. (22) is not incompatible with the differential eqn. (12), because in our treatment there is no initial condition associated with (12) and so this equation does not determine  $c_2$  in terms of  $c_1$ .

**Dr. J. Weiss** (*Newcastle*) said: As we have pointed out in our paper the problem is in certain respects not fully defined and it is essential to take into account the experimental fact that—on account of diffusion and convection—there is *never* a sharp boundary at the top of the band. By paying attention to this fact, diffusion has been taken into account implicitly in our theory. This is not the case in Glueckauf's treatment and it is an important point in our theory.

As we have stated, we have assumed that the development of a chromatogram of two solutes with the solvent is, in general, a continuous process. We have assumed this in view of the experimental results of various workers as there is no evidence to show that one obtains under these conditions a band of constant concentration as postulated by Glueckauf. This is in fact demonstrated clearly even by the experimental work of Coates and Glueckauf.<sup>4</sup> Fig. 2 and 4 of this paper are very instructive and I would like to recommend anyone interested in this problem to consult these Figures and to see for themselves that the "constant band" predicted by this theory is a figment of imagination. These authors have also presented there some theoretical curves. However, in order to show even a rough agreement between theory and experiment they had to choose the arbitrary constants in the theoretical equations in such a way that the "constant band"—which is the main point in Glueckauf's theory—is practically eliminated, i.e., is reduced to such small proportions that it falls well within the region of the experimental error.

I shall be only too pleased to accept Glueckauf's theory if he can produce any experimental evidence in favour of it: that he obviously cannot, and he explains by "disturbing effects" while our theory can at least give an account of the main experimental features.

We find it hard to understand also what Dr. Glueckauf says in the last paragraph of his remark. Diffusion phenomena might be expected to have the effect of spreading out the band and so making the flat region which he predicts more pronounced.

**Dr. J. F. Duncan** (*A.E.R.E., Harwell*) (*communicated*): In order to make an experimental test of the theory developed by Dr. E. Glueckauf, the case shown in Fig. 4 (*d*) of his paper was simulated with the following solutions:

Solution 1: 0.025 N NaNO<sub>3</sub>, 0.1 N KNO<sub>3</sub>, 0.125 N HNO<sub>3</sub>.

Solution 2: 0.15 N NaNO<sub>3</sub>, 0.06 N KNO<sub>3</sub>, 0.04 N HNO<sub>3</sub>.

Assuming the mass action law to obtain we have

$$K_{\text{Na}^+} = \frac{C_{\text{Na}_R}}{C_{\text{H}_R}} \times \frac{C_{\text{H}_S}}{C_{\text{Na}_S}}$$

<sup>3</sup> This Discussion.

<sup>4</sup> *J. Chem. Soc.*, 1947, 1308.

from which

$$\frac{C_{NaR}}{C_R} = \frac{K_{Na+} (C_{NaR}/C_S)}{1 + (K_{Na+} - 1)(C_{NaR}/C_S)}, \quad (1)$$

where the terms have the same meanings as in our paper. Eqn. (1) has the form of a Langmuir equation. Hence

$$b_1 = (K_{Na+} - 1)/C_S,$$

and similarly, for potassium

$$b_2 = (K_{K+} - 1)/C_S.$$

Assuming  $^6 K_{Na+} = 1.52$ , and putting  $K_{K+} = 2.00$  (a reasonable value, estimated from the results of other workers) we get

$$p_{Na+} = 5C_{NaR} \text{ and } p_{K+} = 5C_{KR}.$$

Hence for solution 1,  $p_{Na+} = 0.125$ ,  $p_{K+} = 0.5$ ,

and for solution 2,  $p_{Na+} = 0.75$ ,  $p_{K+} = 0.03$ .

This represents the case when a solution A in the column is followed by a solution C, the characteristic diagram being similar to that shown in Fig. 4 (a). The result of the experiment showed quite clearly that an intermediate concentration plateau forms, with both concentrations higher than A, in the manner shown in Fig. 4 (d).

**Dr. J. Weiss** (*Newcastle-upon-Tyne*) (*communicated*): Dr. Davison has confined his assertions to a special form of the Langmuir isotherm for two solutes, viz.,

$$q_1 = \frac{a_1 c_1}{1 + b_1 c_1 + b_2 c_2}, \text{ etc.},$$

and, furthermore, he makes a somewhat arbitrary choice of the constants. It is obvious that our theory holds, as stated, only when  $q_1$  and  $q_2$  are functions of  $c_1$  and  $c_2$  through the whole course of the development (when both these solutes are present). However, in the Langmuir equations  $q_1$  and  $q_2$  are functions of  $c_1$  and  $c_2$  only if  $b_1 c_1$  and  $b_2 c_2$  are both of the order of 1. It is well known that if this is not the case the equations go over into either (a) the linear isotherms, if  $b_1 c_1$  and  $b_2 c_2 \ll 1$ , or (b)  $q_1$  and  $q_2$  become constant (independent of  $c_1$  and  $c_2$ ), if  $b_1 c_1$  and  $b_2 c_2 \gg 1$ . It is clear that this imposes considerable restrictions on the application of this form of the Langmuir isotherm. Thus it clearly cannot be applied to the displacement development (Tiselius' case) because when the more strongly adsorbed component is present in excess according to this simple form of the Langmuir isotherm the amount adsorbed would become independent of the concentration of this solute. These facts have not been taken into account by Dr. Davison and thus his remarks are hardly of any significance.

**Dr. E. Glueckauf** (*A.E.R.E., Harwell*) (*communicated*): Two matters seem clear from the paper and the contributions of Dr. Offord and Dr. Weiss: (i) that the former has little experience in the application of boundary conditions to hyperbolic differential equations which govern the process concerned (which, by the way, is in close analogy to the propagation of waves in channels); (ii) that the latter tries unsuccessfully to sidetrack the issue by counter attacks which, being without substance, can be easily answered.

No reply has been made concerning Dr. Davison's explicit criticism of eqn. (30), which invalidates the whole of their para. 3 and 4, and it must be assumed that Offord and Weiss concur.

Dr. Offord's explanation "that their treatment only applies when the isotherms have a pronounced curvature" and when the more strongly adsorbed substance is present at higher concentrations is not given expression in any of the boundary conditions used in their paper and it is difficult to escape the impression that it has been an afterthought. In particular, Dr. Offord omits to say that, when

<sup>6</sup> Duncan and Lister, *Chem. and Ind.*, 1949, 24.

he limits application to isotherms of pronounced curvature, and excludes cases where the more adsorbed material (1) is in the minority (which, in terms of the Langmuir isotherm, is equivalent to the conditions  $b_1c_1 + b_2c_2 \gg 1$  and  $c_1^\circ > c_2^\circ$ ), his result approximates more and more to that of my theory. This must be so, because his mathematically correct solution is the point (see my Fig. 3) where the parabolical envelope touches the ordinate at  $p = 1$ . It required exceptionally special assumptions (which the authors do not state in their paper and which do not frequently occur under experimental conditions) before Offord and Weiss's solution would apply even approximately.

Dr. Weiss emphasizes that "in *their* paper, the problem is in certain respects mathematically not fully defined." The reason for this is that they have omitted to introduce an essential boundary condition, namely, the concentration of the original solution  $c_1^\circ$  and  $c_2^\circ$ . This omission is caused by the fact that they impose the condition of continuity of the partial derivatives—a condition which is quite legitimate, and in fact necessary in the elliptic differential equations, but has no justification in equations of the hyperbolic type. And having imposed such a condition they are no longer able to satisfy the initial conditions given by  $c_1^\circ$   $c_2^\circ$ .

That chromatographic conditions (in particular the value of  $c_2$  at  $c_1 = 0$ ) depend on the original concentrations  $c_1^\circ$   $c_2^\circ$  has been shown experimentally and very clearly indeed in the Fig. (4)<sup>6</sup> which Dr. Weiss has examined in so careful and unbiased a manner. The "constant band" which is by no means the main point of my theory, but merely follows from it for certain isotherms, is not and should not be predominant in this case, where the exchange constants  $a_1$  and  $a_2$  differ as little as they do. I also wish to draw attention to the communication by Dr. Duncan concerning experimental data. Dr. Weiss, who has *never* published any chromatographic experiment, has stated "that he will be only too pleased to accept Glueckauf's theory if he can produce any experimental evidence in favour of it." I therefore hope that Dr. Weiss will be pleased to do so.

<sup>6</sup> Coates and Glueckauf, *J. Chem. Soc.*, 1947, 1313.

## ADSORPTION AND SOME CONSTITUTIONAL AND STERIC PROPERTIES

BY L. ZECHMEISTER

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The concept "anchoring group" is proposed and some pertinent experimental approaches are indicated. A report is submitted on the separation of *cis-trans* stereoisomers, and the dependence of the adsorption affinity on molecular morphology is discussed.

Although it is not assumed that a single, correct picture can be given for the adsorption and geometrical orientation of organic molecules on a solid surface, the following concept may serve as a basis of discussion.

Two essential features can be postulated for the simple and stable fixation of an organic solute on an adsorbent: first, its molecules should fit into the cavities of a certain size located on the active surface; and second, adequately oriented forces should be operative between the adsorbent and the adsorbed molecule. These two postulates are, of course, closely interrelated.

Very little is known about the geometrical orientation of adsorbed molecules, but one may speculate that the manner of orientation would influence, among others, the relative behaviour of closely related compounds. If the

orientation be roughly parallel to the surface, it could be perhaps expected that, within a homologous series, a maximum of the adsorption affinity would be reached at a certain chain length. On the other hand, it may be considered that, in the case of a roughly vertical orientation of the adsorbed molecules with reference to the surface, a continuous increase or decrease in the adsorption affinities would be observed by passing stepwise from lower members of the series to some higher ones.

Whatever the orientation may be in a given system, it seems reasonable to assume that not each section of the adsorbed molecule will be equally responsible for the fixation process. On the contrary, we propose that a decisive part will be played by certain atomic groups which are conveniently designated as "anchoring groups." This concept is similar to that in which it is presumed that the fixation of a drug to bacteria takes place by the intermediary of a haptene group (or groups). In favourable instances an anchoring group could be located experimentally by showing that a modification of that particular section of the molecule causes an unusually sharp change in the adsorption affinity.

Perhaps a single example will demonstrate the general lines along which we think that laboratory work may help in collecting material for a more satisfactory discussion of such problems. It was found that the introduction of a methyl group in the  $\alpha$ -position to the sulphur atom of  $\alpha$ -terthienyl markedly increased the adsorption affinity; and that, upon dimethylation, this effect was about doubled. On the other hand, Kofler reported that the adsorbability of some tocol derivatives decreased upon methylation near the phenolic OH group, although this substitution had but little effect in a more remote position to the hydroxyl mentioned. In the case of thiophene rings the introduced methyl group seems to have been involved in the fixation process but the anchoring group of the tocol derivative was probably the phenolic hydroxyl whose function is sterically hindered by the presence of adjacent methyl groups.

While manifold possibilities are open for experimentation in the direction just outlined, our problem changes its character entirely when we pass from chemical to stereochemical considerations. Then, in a way, the situation appears to be simplified, since in stereochemistry the dependence of the adsorption affinity on the overall shape of the molecules is to be considered, and not the presence or absence of certain functional groups. The compounds which are suitable for such investigations should possess numerous stereo-isomeric forms whose respective morphological types should vary as widely as possible. This postulate is unequally fulfilled in various stereo-isomeric sets; for example, the epimerization of a sugar would not essentially alter the overall shape of its molecules.

The conditions for studies of this kind are especially favourable in the field of the natural and synthetic polyenes, which compounds possess a long, conjugated carbon-carbon double-bond system in an open chain. Their structure is "morphologically sensitive" to spatial variations. Although the ordinary or all-*trans* form (Fig. 1) which shows a rod-like general shape is not greatly modified by a single *trans*→*cis* rotation which occurs near an end of the system, it undergoes a radical change and is converted into a V-like pattern when such a spatial re-arrangement takes place at or near the centre (II). On the other hand, in the course of continued *trans*→*cis* rotations, after having passed through several bent forms, a straightening-out takes place. Thus, the molecules of the resulting poly-*cis* compound show a rod-like overall shape (III) which is morphologically similar to that of the all-*trans* molecules (I).

As is well known, the respective all-*trans* forms of the polyene hydrocarbons ( $C_{40}H_{56}$ ),  $\alpha$ -carotene,  $\beta$ -carotene,  $\gamma$ -carotene, and lycopene, can

be easily converted into a mixture of stereo-isomers containing mainly compounds with mono-*cis* and di-*cis* configurations. Such a mixture is resolved chromatographically, whereupon some insight into the respective configurations can be gained by means of appropriate spectroscopic methods.

Whereas any manner of bending of an all-*trans* carotene molecule causes a displacement of the main extinction maxima towards shorter wavelengths in the visible spectral region, the simultaneously occurring alteration in the adsorption affinity does not follow such a simple rule. It was found for each of the three carotenes, for example, that a stereo-isomer which very probably contains a single, peripherally-located *cis* double bond, is adsorbed above the corresponding all-*trans* compound in the Tswett column. In contrast, those spatial forms of the carotenes which possess a centrally-located *cis* double bond (and in some instances another *cis* bond) show considerably weaker adsorbability than the all-*trans* isomer. Thus, we have the following chromatographic sequence:

- |          |   |                               |
|----------|---|-------------------------------|
| (Top)    | Peripheral mono- <i>cis</i> carotene      | } essentially straight types. |
|          | All- <i>trans</i> carotene                |                               |
| (Bottom) | Central mono- <i>cis</i> carotene, etc. : | essentially bent type.        |

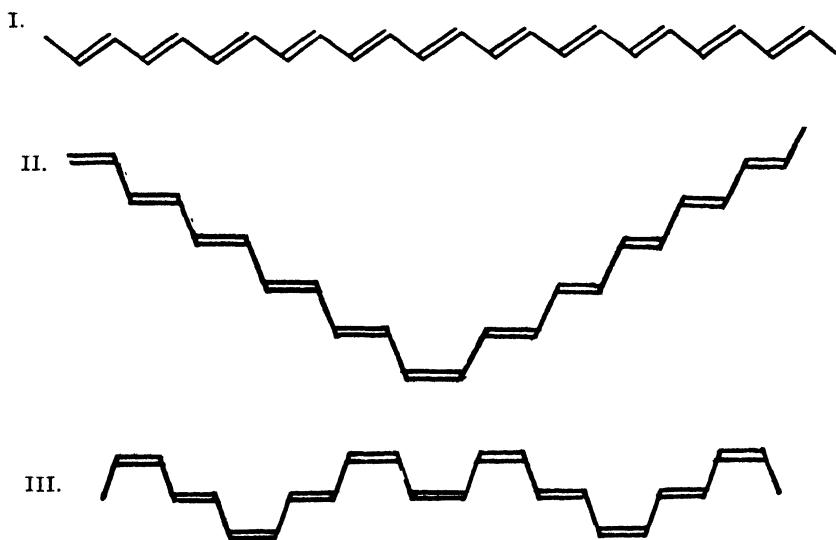


Fig. 1.

Typical spatial forms of a long, conjugated double bond system in an open chain :  
I, all-*trans* ; II, central mono-*cis* ; and III, poly-*cis* (penta-*cis*).

It should be noted in this connection that the adsorption affinity of a monohydroxy- $\beta$ -carotene, viz., all-*trans* cryptoxanthin ( $C_{40}H_{55}.OH$ ), undergoes the changes just described when stereo-isomerized *in vitro*; hence, in this instance the presence of a single hydroxyl group has no decisive influence on the relative adsorbabilities of the respective spatial forms.

In contrast, each *cis* isomer so far observed of the analogous dihydroxy compounds, viz., zeaxanthin and lutein ( $HO.C_{40}H_{54}.OH$ ), is located in chromatograms above the corresponding all-*trans* zone. Thus, the outstanding features of this *trans*  $\rightarrow$  *cis* isomerization are weakening of the colour and an

increase in the adsorption affinity. One could suppose that in these stereochemical sets both hydroxyl groups would participate in the anchoring process, and that the decrease of the distance between them, as caused by bending of the molecule, would promote fixation. However, it is also possible that such spatial forms of the dihydroxycarotenes do exist which show decreased adsorption affinities, as compared with the all-*trans* form; but for some reason they do not appear in appreciable quantities in the stereo-isomeric mixtures which can be obtained by current methods.

Some representatives of a different type of stereo-isomeric hydrocarbons, viz., poly-*cis*  $\gamma$ -carotenes and poly-*cis* lycopenes, which so far we were unable to prepare in the laboratory, could be isolated from some plant materials. They contain four to seven of their double bonds in *cis* configuration. If we pass from an all-*trans* compound to either of its poly-*cis* forms, then the remarkable weakening in the colour runs parallel with a substantial decrease in the adsorption affinity. A similar parallelism is also observed within the subclass of the poly-*cis* lycopenes, whose chromatographic sequence and spectral sequence are identical. With reference to both of these physical characteristics the individual differences are much smaller within the class of the poly-*cis* isomers than between either poly-*cis* form and all-*trans* lycopene.

Before closing these considerations, we should stress that the described variations in the adsorption affinity which are a function of morphological changes, are of the same order of magnitude as analogous effects caused by reasonably-chosen structural conversions. For example, a solution containing several spatial forms of both  $\alpha$ -carotene and  $\beta$ -carotene gave the following chromatographic sequence on calcium hydroxide, when developed with petroleum ether:

(Top)	<i>Neo</i> - $\beta$ -carotene <i>V</i>
	<i>Neo</i> - $\alpha$ -carotene <i>U</i>
	All- <i>trans</i> $\beta$ -carotene
	<i>Neo</i> - $\alpha$ -carotene <i>V</i>
	<i>Neo</i> - $\beta$ -carotene <i>B</i>
	<i>Neo</i> - $\beta$ -carotene <i>E</i>
	<i>Neo</i> - $\alpha$ -carotene <i>W</i>
	<i>Neo</i> - $\beta$ -carotene <i>F</i>
	All- <i>trans</i> $\alpha$ -carotene
(Bottom)	<i>Neo</i> - $\alpha$ -carotene <i>B</i>

Evidently, the weaker adsorption affinity of all-*trans*  $\alpha$ -carotene (containing 10 conjugated double bonds) as compared with that of all-*trans*  $\beta$ -carotene (11 such bonds) can be compensated and even overruled by a suitable adjustment of the molecular form.

Furthermore, an increase of the adsorption affinity several times stronger than that caused by the reaction,  $\alpha$ -carotene  $\rightarrow$   $\beta$ -carotene, is obtained when we convert a poly-*cis* compound into the corresponding all-*trans* form.

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# CHROMATOGRAPHY OF COLOURLESS SUBSTANCES AND THE RELATION BETWEEN CONSTITUTION AND ADSORPTION AFFINITY

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A method for the chromatographic separation of colourless substances based on the use of fluorescent adsorbents is described. Relations between adsorption affinity and chemical constitution of azobenzene and stilbene derivatives are demonstrated. The dependence of adsorption affinity on the nature of the adsorbent and solvent was investigated. Water soluble salts like copper sulphate or zinc sulphate were found to be useful adsorbents for the chromatographic separation of azobenzene compounds.

**Chromatography of Colourless Substances.**<sup>1</sup>—The adsorption zones of colourless substances can be followed by the use of fluorescent adsorbents. Ultra-violet light of a wavelength absorbed by the adsorbed substance is employed. The part of the column containing adsorbed substance then appears as a dark band. Adsorbents can be made to fluoresce by the addition of small quantities of irreversibly adsorbed fluorescing compounds. Morin is very satisfactory for alumina, magnesium oxide and calcium carbonate, and berberine for silica. Salicylic acid and 2-hydroxynaphthalene-3-carboxylic acid can also be used.

**Experimental.**—A very convenient method for the preparation of a fluorescent column consists in the mixing of the adsorbent with 2 % to 5 % of finely powdered luminous paint. This method is generally applicable.

Calcium sulphate dihydrate, prepared by the precipitation of calcium chloride with the calculated quantity of sulphuric acid at 80°–90° C can be converted into a valuable adsorbent for colourless compounds, and also, incidentally, an excellent one for coloured ones. Heating at temperatures between 150° and 200° C yields three different products: the hemihydrate has an activity equivalent to that of alumina IV; the soluble anhydrous salt can be obtained in two grades, one equivalent to alumina III and the other to alumina II. The product obtained by heating above 250° is only slightly active and is of no practical value. The large adsorption affinity of the soluble anhydrite is very likely to be due to numerous faults in the crystal lattice.

The fluorescent chromatograms were illuminated with light of 366 m $\mu$  from a mercury vapour lamp and an appropriate filter. For compounds which absorb at shorter wavelengths, a Chlorfilter was used with windows of UGS glass (Schott, Jena) which transmits only the Hg line at 253.7 m $\mu$ . In our most recent experiments on the separation of colourless compounds, the following apparatus was used. Light from an electric arc (iron electrodes) was passed through a slit 35 mm. long and 3–4 mm. wide, a quartz lens 5 cm. thick, and a quartz or rock salt prism 50 mm. high. The resulting line spectrum (length of lines 50 mm.) was projected onto a fluorescent screen; after focusing, the fluorescent screen was replaced by a cardboard sheet on which the position of the spectral lines in the ultra-violet spectrum had been marked. The adsorption column, contained in a quartz tube, was placed in front of the cardboard and moved until the optimum contrast was obtained making the bands of the chromatogram clearly recognizable. It is advisable to employ a combination of adsorbent and fluorescent compound which does not continue to emit light after illumination (luminous paint, N<sub>4</sub> green I, Francke, Frankfurt, was used).

<sup>1</sup> Brockmann and Volpers, *Ber.*, 1947, **80**, 77; 1949, **82**, 95. Sease, *J. Amer. Chem. Soc.*, 1947, **69**, 2242.

**Appearance of Zones.**—There are two ways in which the zones can be made apparent: (i) in the case of adsorbents rendered fluorescent by adsorbed morin or berberine, the zones become apparent through extinction of the fluorescence by the adsorbed compound; (ii) adsorbents which have been mixed with luminous paint behave differently. In this case the zones appear as follows. The particles of the adsorbent allow the light to penetrate the column to a certain extent and fluorescence excited there is emitted. Actual adsorption of the light is stronger at places where the adsorbed compound is present, so that the fluorescing material, in this case the luminous paint particles, is not as strongly excited as in those places where the column is free: luminous paint particles thus act as small fluorescent screens.

**Experimental.**—That the outer layer of adsorbent allows light to penetrate is shown as follows. A test-tube, the diameter of which is such that it fits in the chromatographic tube with 3–5 mm. clearance all round, is filled with luminous paint and placed in the tube. The space between is filled with adsorbent and a colourless substance is adsorbed onto it. On illumination with ultra-violet light the zone appears surprisingly clearly. This filter action of the outer layer of the column can also play a part in the appearance of the zones when adsorbent materials containing adsorbed morin or berberine are employed. In this case each particle of adsorbent acts as a small fluorescent screen.

Whether an adsorbed colourless substance is detected through extinction of the fluorescence or through the filter effect can be decided in some cases by comparing the behaviour of an adsorbent + luminous paint with that of the adsorbent + morin or berberine. Table I gives the results of such a comparison.

TABLE I

			Al <sub>2</sub> O <sub>3</sub> —Luminous paint	Al <sub>2</sub> O <sub>3</sub> —Morin
Benzaldehyde	..	..	Non-visible	Visible
<i>p</i> -Tolylaldehyde	..	..	"	"
Acetophenone	..	..	"	"
Anisaldehyde	..	..	"	"

A mercury lamp with a filter allowing the transmission of light of wavelength 360–370 mμ was used. Since the above compounds hardly absorb at 365 mμ the zones do not appear when adsorbent + luminous paint is employed. The appearance of the zones when adsorbent + morin is used must therefore be due to extinction of fluorescence. The effectiveness of the extinction is greater in the case of morin than that of berberine. Thus the phenacyl esters of aliphatic carboxylic acids, which hardly absorb at 365 mμ, are visible with light of this wavelength on Al<sub>2</sub>O<sub>3</sub>-morin but not on silica-berberine.

**Relations Between Constitution and Adsorption Affinity.**—The adsorption affinity, that is the firmness with which organic compounds are adsorbed from non-aqueous solvents *inter alia*, depends on the nature of the skeleton and of the functional groups of the adsorbed compound.

In order to find the effect of the functional groups on adsorption affinity, several compounds containing the same basic skeleton but differing only in one functional group were investigated. The first experiments were carried out with *p*-substituted stilbenes and azobenzenes. These compounds were adsorbed on Al<sub>2</sub>O<sub>3</sub> from benzene or from carbon tetrachloride. Since stilbene and azobenzene under these conditions are only very weakly adsorbed, the adsorption affinity of the derivatives can be taken to be almost entirely dependent on the nature of the substituents. The functional groups in Table II are arranged in order of decreasing adsorption affinity.



Compounds separated by a horizontal dash could be separated, the others were not fully separable. The two series are in almost complete agreement with each other. Differences are only found in the three unnumbered acyl derivatives on the right-hand side: those in the azobenzene series lie higher than those in the stilbene series; the order, however, remains the

TABLE II

$R = C_6H_5.CH:CH.C_6H_4-$	$R = C_6H_5.N:N.C_6H_4-$
$R-COOH$	$R-COOH$
$R-CONH_2$	
$R-OH$	$R-OH$
	$R-NH-Ac$
	$R-O-Ac$
$R-NH_2$	$R-NH_2$
	$R-O-Bz$
$R-NH-Ac$	
$R-O-Ac$	
$R-COOCH_3$	$R-COOCH_3$
$R-N(CH_3)_2$	$R-N(CH_3)_2$
$R-NO_2$	$R-NO_2$
$R-OCH_3$	$R-OCH_3$
$R-H$	$R-H$

Ac =  $CH_3.CO-$     Bz =  $C_6H_5.CO-$

same. This difference is probably due to the fact that the azobenzene derivatives were adsorbed from benzene and the stilbene derivatives from carbon tetrachloride. The solubility of the azobenzene derivatives in carbon tetrachloride was unfortunately too small for adsorption experiments to be carried out using this solvent.

**Dependence of Adsorption Affinity on the Adsorbent.**—In the adsorption of organic compounds from non-aqueous solvents only, certain points on the adsorbent particle (the so-called active points) play a part. These are mainly at positions where the crystal lattice is faulty. The adsorption activity of an adsorbent therefore does not only depend on its chemical nature, but also on the nature of its crystal lattice.  $\alpha-Al_2O_3$  does not adsorb whereas  $\gamma-Al_2O_3$  and Böhmite are very effective as adsorbents.  $CaSO_4$ , like the "soluble" anhydrous salt, possesses the same crystal lattice as the hemihydrate; it shows good adsorption properties, and is, in the light of our experience, very efficient in the separation of hydroxy-anthraquinones. As the lattice of the soluble anhydrite is changed into that of the insoluble anhydrite by heating to  $250^\circ$ , the capacity for adsorption falls very considerably.

We have previously shown that  $\gamma-Al_2O_3$  can be obtained in five different grades (by treatment with water vapour or by regulated heating) which can differentiate by adsorption of azo dyes under standardized conditions. The employment of  $Al_2O_3$  with different and reproducible activities has proved so useful that we have attempted to prepare graded specimens of other adsorbents. By varying its water content, bentonite was obtained in five grades of activity, silica gel and precipitated silica in three,  $CaSO_4$  and  $MgO$  also in three, and  $CaCO_3$  in two different grades. The standardization of these adsorbents was carried out using the same dyes as in the case of alumina. In the course of these experiments we have tried to find out whether, with the same solvents, our test dyes were adsorbed in the same order as on  $Al_2O_3$ . The following experiments show this not to be the case.

**Adsorption Sequence of Azo Dyestuffs in Columns of Different Adsorbents.**

DYESTUFFS: *p*-hydroxyazobenzene, *p*-amino-azobenzene, Sudan Red ( $(o\text{-CH}_3)\cdot\text{C}_6\text{H}_4\cdot\text{N}:\text{N}\cdot\text{C}_6\text{H}_4(o\text{-CH}_3)\cdot\text{N}:\text{N}\cdot\text{C}_{10}\text{H}_6\cdot\text{OH}$ , Sudan Yellow  $\text{C}_6\text{H}_5\cdot\text{N}:\text{N}\cdot\text{C}_{10}\text{H}_6\cdot\text{OH}$ , *p*-methoxyazobenzene.

SOLVENT: Benzene-Petrol ether, 1/4.

The most strongly adsorbed dyestuff is listed at the top of each column, the least adsorbed at the bottom.

$\text{Al}_2\text{O}_3$	$\text{SiO}_2$	$\text{MgO}$
Hydroxyazobenzene	Sudan Red	Hydroxyazobenzene
Amino-azobenzene	Oxyazobenzene	Sudan Yellow, Sudan Red
Sudan Red	Amino-azobenzene	Amino-azobenzene
Sudan Yellow	Sudan Yellow	Methoxyazobenzene
Methoxyazobenzene	Methoxyazobenzene	

$\text{CaSO}_4$	$\text{CuSO}_4$
Hydroxyazobenzene, Sudan Yellow	Amino-azobenzene
Amino-azobenzene	Sudan Red
Sudan Red	Hydroxyazobenzene
Methoxyazobenzene	Sudan Yellow
	Methoxyazobenzene

**Effect of the Acid or Alkaline Character of the Adsorbent on the Adsorption Sequence.**—The adsorption sequence of acidic or basic dyes is influenced by the acidity or alkalinity of the adsorbent as the following observations show.

SOLVENT: Benzene-Petrol ether, 1/4.

$\text{Al}_2\text{O}_3$ alkaline	$\text{Al}_2\text{O}_3$ treated with HCl
Hydroxyazobenzene	Hydroxyazobenzene
Amino-azobenzene	Sudan Red
Sudan Red	Amino-azobenzene
Sudan Yellow	Sudan Yellow
Methoxyazobenzene	Methoxyazobenzene

$\text{SiO}_2$ treated with NaOH	$\text{SiO}_2$ treated with HCl
Sudan Red	Sudan Red
Amino-azobenzene	Hydroxyazobenzene
Hydroxyazobenzene	Amino-azobenzene
Sudan Yellow	Sudan Yellow
Methoxyazobenzene	Methoxyazobenzene

**Effect of Different Solvents on the Adsorption Sequence.**—The firmness with which a compound is adsorbed from a non-aqueous solvent depends on the nature of the solvent in two ways: (i) the solvent tends to occupy the active points of the adsorbent; (ii) the solvent reacts with the dissolved substance (solvation). Both these effects are negligible with non-polar solvents such as petrol ether, cyclohexane, carbon tetrachloride and benzene, but considerable with polar solvents. The effect of various solvents on the adsorption sequence of dyestuffs is shown in the following results.

ADSORBENT :  $\text{Al}_2\text{O}_3$ Benzene-Petrol ether, 1/4Carbon tetrachloride

Hydroxyazobenzene  
Amino-azobenzene  
Sudan Red  
Sudan Yellow  
Methoxyazobenzene

Ether

Sudan Yellow, Hydroxyazobenzene  
Sudan Red  
Amino-azobenzene  
Methoxyazobenzene

Chloroform

Hydroxyazobenzene  
Amino-azobenzene  
Sudan Yellow  
Sudan Red  
Methoxyazobenzene

Tetrahydrofuran, Dioxan, Cyclohexanone

Hydroxyazobenzene  
Sudan Red, Sudan Yellow  
Amino-azobenzene  
Methoxyazobenzene

ADSORBENT :  $\text{CaSO}_4$ Benzene-Petrol ether, 1/4

Sudan Yellow, hydroxyazobenzene  
Amino-azobenzene  
Sudan Red  
Methoxyazobenzene

Ether

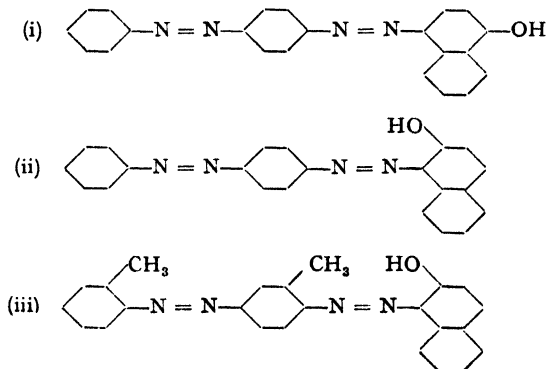
Sudan Yellow, hydroxyazobenzene  
Amino-azobenzene  
Sudan Red  
Methoxyazobenzene

Chloroform

Sudan Yellow, hydroxyazobenzene  
Amino-azobenzene  
Sudan Red  
Methoxyazobenzene

There does not seem to be a relationship between the adsorption sequence and the solubility of the test dyestuffs.

It is remarkable that Sudan Yellow and hydroxyazobenzene are adsorbed equally firmly from ether. A similar effect with ether as solvent was observed in the case of the three following Sudan dyes.

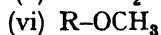
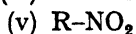
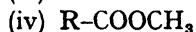
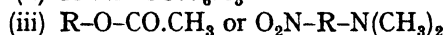
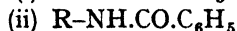
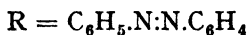


These compounds are adsorbed on  $\text{Al}_2\text{O}_3$  from benzene-light petroleum 1/4 in the order (i), (ii), (iii), but from ether in the order (ii), (iii), (i).

Even though no satisfactory explanation can at the moment be given of the effect of the solvent on the adsorption sequence, our observations lead to the following procedure. In the case of a complex mixture which cannot be separated by the use of one adsorbent and one eluent, it has been suggested that separation may be effected by adsorption from a non-polar solvent onto a strong adsorbent, followed by elution with a series of solvents of increasing adsorption affinity. We believe that this procedure when applied to compounds such as our dyestuffs, which in the adsorption sequence are so dependent on the nature of the solvent, is not as satisfactory as a procedure, which in our experience has proved to be of value, and which involves repeated chromatography of the mixture from one non-polar solvent on several adsorbents of varying activity.

**The Adsorption Sequence which is independent of both Adsorbent and Solvent.**—The marked dependence of the adsorption sequence of our dyestuffs on the nature of the solvent is perhaps due to variations in the solvation of the hydroxyl and amino groups present. Furthermore, it is possible that, depending on the nature of the solvent, the dyestuffs are adsorbed partly in the azobenzene form, partly in the tautomeric quinone-imine form.

These complications are avoided if the following compounds are used. The adsorption affinity decreases as one goes down the series.



Instead of dyestuff (iii) which will be partly hydrolyzed on alkaline adsorbents, *p*-bromo-*p*'-dimethylamino-azobenzene was employed. This compound, as a result of polarized adsorption, forms a bluish-violet zone. These compounds were always adsorbed in the same order from the following solvents on the following adsorbents.  $Al_2O_3$ : benzene-petrol ether 1/4, ether, chloroform. From ether, separation of (i) and (ii) is incomplete.  $SiO_2$ : benzene-petrol ether 1/4, ether, chloroform.  $CaSO_4$ : benzene-petrol ether 1/4, benzene, ether.  $MgO$ : benzene-petrol ether 1/4.  $CuSO_4$ : benzene-petrol ether.

From the above results we believe that we may draw the following conclusions concerning the relations between constitution and adsorption. The adsorption affinity of a compound is practically an additive function of the adsorption affinities of the basic skeleton and of the functional groups only when there is no possibility of tautomerism and when solvation by the solvent employed does not occur. The above-mentioned series can be taken to represent the relative adsorption affinities of the functional groups. Deviations from the above sequence are to be observed only when the adsorption behaviour of the functional groups is very similar. Strain<sup>2</sup> has drawn similar conclusions, using carotinoids. The knowledge that adsorption affinity depends largely on the number and nature of the functional groups only when the skeleton of the molecules is not very different enables one to predict in many cases whether chromatographic separation is possible.

<sup>2</sup> *J. Amer. Chem. Soc.*, 1948, 70, 588.

**Water-soluble Salts as Adsorbents.**—Anhydrous copper sulphate has shown itself to be particularly well suited to the separation of azobenzene derivatives. The following azobenzene derivatives were adsorbed from benzene–light petroleum 1/4 and were eluted with the same solvent mixture or with benzene alone: the compounds were found to be adsorbed in the following order of decreasing adsorption affinity.

$R = -C_6H_4 \cdot N:N \cdot C_6H_4 -$	Colour of the zones
(i) $H-R-NH_2$	Flesh-coloured
(ii) $(CH_3)_2N-R-N(CH_3)_2$	Bluish red
(iii) $H-R-N-(CH_3)_2$	Purple
(iv) $Br-R-N(CH_3)_2$	Blue
(v) $H-R-NH \cdot CO \cdot CH_3$	Reddish brown
(vi) $H-R-NH \cdot CO \cdot C_6H_5$	Pale red

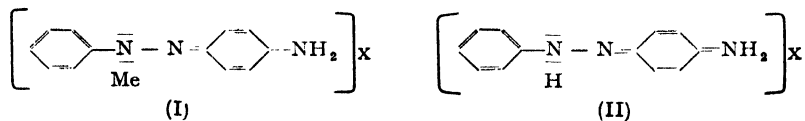
The following azobenzene derivatives, which cannot be separated on alumina are easily separated on anhydrous copper sulphate.

- (1)  $R-COOCH_3$ ,  $R-N(CH_3)_2$ .  $R-N(CH_3)_2$  is well adsorbed from benzene onto copper sulphate,  $R-COOCH_3$  on the other hand forms an easily eluted yellow zone.
- (2)  $R-NH_2$ ,  $R-NH \cdot CO \cdot C_6H_5$ .  $R-NH_2$  is firmly adsorbed onto  $CuSO_4$  from benzene as a flesh-coloured zone from which  $R-NH \cdot CO \cdot C_6H_5$  is easily separated by elution.
- (3)  $R-NH_2$ ,  $R-O-CO \cdot CH_3$ .  $R-NH_2$  is adsorbed from benzene much more firmly than  $R-O-CO \cdot CH_3$ .
- (4)  $R-NO_2$ ,  $R-N(CH_3)_2$ . The purple zone of  $R-N(CH_3)_2$  is much more firmly adsorbed than the orange zone of the nitro compound.

Anhydrous zinc, manganese, aluminium and magnesium sulphates can also be used for the separation of azobenzene derivatives.

We believe that it is probable that the addition compounds of the type (I) analogous with salts of *p*-amino-azobenzene (II) are formed on the surface of the adsorbent.

The above-mentioned salts, particularly aluminium sulphate, can be used for the separation of other compounds such as hydroxy-anthraquinones. Compounds that are very firmly adsorbed onto these salts can be isolated simply by dissolving the inorganic material in water and extracting the organic compounds with a suitable solvent.



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# THE FUNCTION OF ADSORBENT ACTIVITY IN THE CHROMATOGRAPHIC SEPARATION OF CERTAIN ANTHRAQUINONE COMPOUNDS

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The activity of adsorbents used in chromatography with organic solvents is easily controlled by preliminary incorporation of water in the adsorbent. The effect on the band movement of simple anthraquinone compounds in toluene on activated alumina and unactivated magnesium carbonate is described. The conditions for preparing activated alumina by heating between 250° and 500° C have considerably less effect on the behaviour than has the deactivation treatment. The band movement measurements were made under satisfactory comparative conditions but are affected by several errors; in particular the greater solvent movement in mid-column than at the periphery renders values obtained at the periphery 11 % low.

The distribution of the anthraquinone compounds between solvent and partially deactivated alumina is independent of concentration. The length of the initial chromatograph band and the mean rate of band movement are in satisfactory accord with theory. The considerable band widening in passage through a column is independent of the adsorption, little affected by the rate of solvent flow and may be caused by non-uniformity of flow through the interstitial capillary spaces; its effect on the separation of a mixture causes the various bands to have approximately equal width at the same position in the column.

The relation between constitution and chromatographic behaviour of simple anthraquinone compounds, largely influenced by the conditions used, is briefly described.

The successful application of adsorption chromatography ultimately depends on the empirical selection of operating conditions, if for no other reason than that the composition of the mixtures examined is rarely fully known until the separation has been achieved. Various methods have been described for the partial deactivation of adsorbents<sup>1-5</sup> and it is the purpose of this paper to draw attention to the simplicity of this operation and the wide range of control exercised on the band movement, enabling this variable to be systematically applied in selecting the operating conditions. To illustrate the behaviour, band movement measurements have been made using the highly coloured simple anthraquinone compounds in toluene solution; these conditions do not necessarily represent the optimum for such separations, for which their fairly low solubility is often a controlling factor. The effect on activated alumina and magnesium carbonate adsorbents is described; it has been applied to other adsorbents, but with the wide range of control obtained on deactivation these adsorbents cover most of the activity range required when the separation is carried out from organic solvent solution. The effect of variation in the conditions for the activation of alumina is shown to have much less effect on the behaviour than has the subsequent deactivation.

Partial deactivation of activated alumina gives a distribution between adsorbent and solvent which is substantially independent of the concen-

<sup>1</sup> Zechmeister and Cholnoky, trans. Bacharach and Robinson. *Principles and Practice of Chromatography*. (Chapman & Hall, London, 1941, p. 48.)

<sup>2</sup> Brockmann and Schodder, *Ber.*, 1941, **74**, 73.

<sup>3</sup> Müller, *Verb. Ver. Schweiz. Physiol.*, 1942, **21**, 29; *Helv. chim. Acta*, 1943, **26**, 1945; 1944, **27**, 404.

<sup>4</sup> Schroeder, *Ann. N.Y. Acad. Sci.*, 1948, **49**, 204.

<sup>5</sup> Stewart and I.C.I. Ltd., *Brit. Pat.* 565,405.

tration and under these conditions the mean band movement and the length of initial band are shown to be in agreement with the theory described by LeRosen <sup>6</sup> and others.<sup>7-10</sup> Conditions affecting the widening of bands, often a limiting factor in the chromatographic separation, are considered.

### Theoretical

The symbols used have the following significance:

$i$ .. ..	Weight of interstitial solvent per unit length of the column.
$m$ .. ..	Weight of adsorbent per unit length of the column.
$K$ .. ..	Distribution constant = the weight of solute adsorbed per gram of adsorbent/equilibrium concentration of solution in gram per gram, this value being substantially constant under the conditions employed.
$K'$ .. ..	Distribution constant prevailing in unit length of the column, $K' = Km/i$ .
$c$ .. ..	Concentration of solution.
$w$ .. ..	Weight of solution.
$x$ .. ..	Distance of any point in the column measured from the beginning (top) of the column.
$D$ .. ..	Distance the developing solvent has moved in the column.
$c_0$ .. ..	Initial concentration of solution added to column.
$x_0$ .. ..	Length of the initial band formed by adding $w_0$ to column. $x_0^\alpha, x_0^\beta$ referring to initial lengths of components $\alpha$ and $\beta$ .
$D_0$ .. ..	Length of column occupied by interstitial solvent of weight $w_0$ .
$D_1^{\alpha\beta}$ .. ..	Distance the developing solvent requires to move to separate two bands starting from $x_0^\alpha$ and $x_0^\beta$ .
$R$ .. ..	Displacement of zone on column/displacement of solvent in column $R = dx/dD$ . $R_L$ for the leading edge of a band; $R_F$ for the following edge; $R_M$ for the mean of $R_L$ and $R_F$ .
$\% R_M$ .. ..	$R_M$ expressed as a percentage of the solvent movement.
$\% H$ .. ..	Extent of water deactivation; ml. water addition per 100 g. adsorbent, the latter including any moisture initially present.
$R_L/R_M$ .. ..	A measure of the widening of a band edge in passage through the column.

As LeRosen <sup>6</sup> and others <sup>7-10</sup> have shown, where the distribution between adsorbent and solvent is independent of concentration and is rapidly established, the conditions for the formation and separation of the bands can be evaluated.

If a solution of concentration  $c_0$  of a pure compound is added to a column in amount  $w_0$  equal to length  $D_0$  of interstitial solvent, the ratio of interstitial solvent to adsorbent in unit length of the column being  $i/m$ , then the solute is adsorbed until it reaches equilibrium with the initial concentration of the solution and a band of length  $x_0$  is formed such that

$$x_0 c_0 + K' x_0 c_0 = D_0 c_0 \text{ or } x_0 = D_0 / (1 + K') = D_0 / (1 + Km/i) \quad (1)$$

If pure solvent is now allowed to flow through the column then the band moves down at a fraction of the rate of linear movement of the solvent equal to the fraction of the total substance present in the interstitial solvent. The movement of the band relative to the linear movement of solvent through the column  $R$  is given by

$$R = x_0/D_0 = 1/(1 + K') = 1/(1 + Km/i), \quad (2)$$

$$\text{or} \quad K' = (1 - R)/R \text{ and } K = [(1 - R)/R] i/m \quad (3)$$

<sup>6</sup> LeRosen, *J. Amer. Chem. Soc.*, 1947, **69**, 87.

<sup>7</sup> De Vault, *J. Amer. Chem. Soc.*, 1943, **65**, 532.

<sup>8</sup> Weiss, *J. Chem. Soc.*, 1943, 297.

<sup>9</sup> Glueckauf, *J. Chem. Soc.*, 1947, 1302.

<sup>10</sup> Weil-Malherbe, *J. Chem. Soc.*, 1943, 303.

The movement of the chromatograph bands through a column is conveniently expressed<sup>11</sup> as the relative movement  $R$ , which is given here as a percentage of the solvent movement. The value depends on the ratio of solvent to adsorbent  $i/m$  for the particular column, so that the results obtained from different columns require to be calculated on the basis of eqn. (2) and (3) to correspond with a particular value of  $i/m$ .

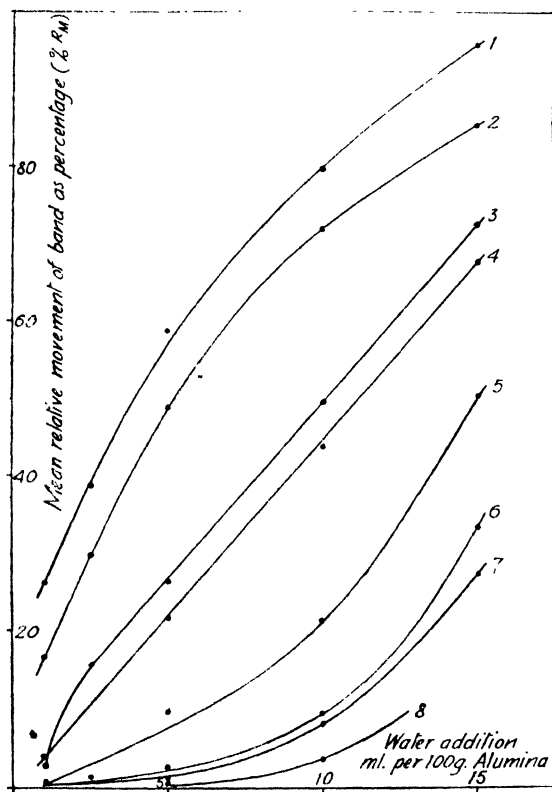


FIG. 1.—Effect of water deactivation treatment applied to activated alumina on the band movements of anthraquinone compounds in toluene.

- Curve 1. 1-Chloroanthraquinone.  
 " 2. 1-Methylaminoanthraquinone.  
 " 3. 1-Aminoanthraquinone.  
 " 4. 1-Dimethylaminoanthraquinone.  
 " 5. 1-Amino-4-methylaminoanthraquinone.  
 " 6. 2-Aminoanthraquinone.  
 " 7. 1 : 4-Diaminoanthraquinone.  
 " 8. 1 : 4 : 5-Triaminoanthraquinone.

Under the above conditions the band should move down the column without changing its length, the small region in front and rear where adsorption and desorption occur remaining constant. The position of the following and leading edges of a band is given by

$$\text{Following edge : } x = x_0 D/D_0 \quad . \quad . \quad . \quad (4)$$

$$\text{Leading edge : } x = x_0 + x_0 D/D_0 \quad . \quad . \quad . \quad (5)$$

<sup>11</sup> LeRosen, *J. Amer. Chem. Soc.*, 1942, **64**, 1905.



If a second component is present in the initial solution and there is no mutual interference in the distribution, the bands will draw apart and commence to separate when the following edge of the faster reaches the leading edge of the slower

$$x_o^\beta D_s/D_o = x_o^\alpha + x_o^\alpha D_s/D_o \text{ or } D_s^{\alpha\beta}/D_o = x_o^\alpha/(x_o^\beta - x_o^\alpha), \quad (6)$$

the actual position on the column being given by  $R^\beta D_s$ .

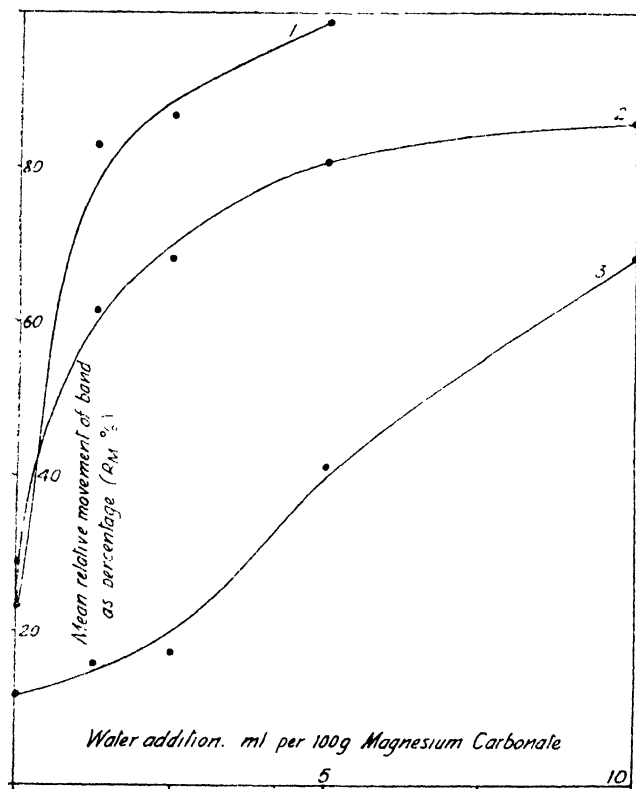


FIG. 2.—Effect of water deactivation treatment applied to unactivated magnesium carbonate on the band movement of anthraquinone compounds in toluene.

- Curve 1. 1-Hydroxy-4-*p*-toluidinoanthraquinone.  
 „ 2. 1 : 4 : 5-Triaminoanthraquinone.  
 „ 3. 1 : 4 : 5 : 8-Tetraminoanthraquinone.

The solvent flow required to produce the initial separation should depend on the ratio of the lower  $R$  value to the difference in  $R$  values, while the position at which this separation occurs and the actual rate at which the bands subsequently move apart depend on the value of  $R$ .

Certain complications limit the simple application of the above relations. The leading edge of a band moves considerably (20 %–30 %) faster than the following edge and the diminishing concentration at the band edges increases the difficulty of measuring the band movement and determining the position of separation. The bands move at a different rate in mid-column than at the periphery, probably 16 % faster in the columns considered below.

## Experimental

**The Control of Adsorbent Activity by Partial Deactivation.**—The preferred method of deactivation consists in adding liquid water to the adsorbent, shaking to distribute the soft wet portion initially formed and mixing for 2 hr., conveniently by rotating the container. The addition necessary causes little or no change in the handling properties of the adsorbents; the method is easy to carry out and gives a reproducible behaviour. The deactivation is expressed as ml. water addition per 100 g. adsorbent, abbreviated to %  $H$ , the weight of adsorbent including any moisture initially present.

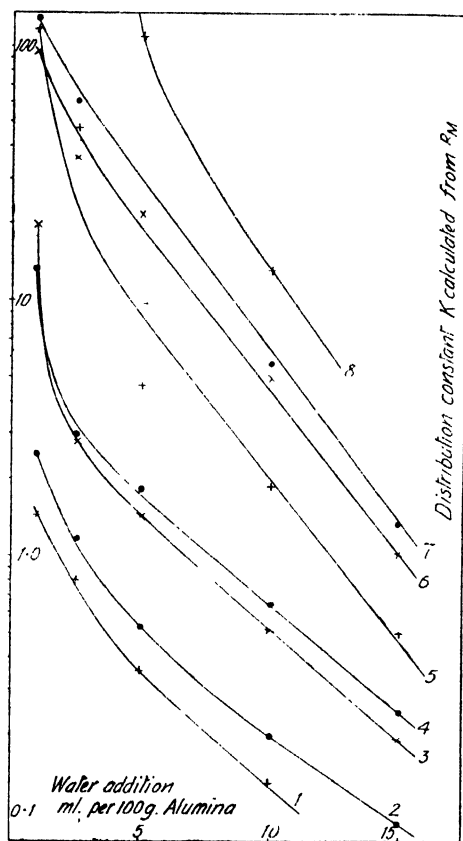


FIG. 3.—The effect of water deactivation treatment applied to activated alumina on the distribution constant of anthraquinone compounds between toluene and adsorbent. The curves are numbered as in Fig. 1.

The effect of partial deactivation on the band movement of simple anthraquinone compounds in toluene on activated alumina (Type O; P. Spence & Sons Ltd.) and on unactivated magnesium carbonate (Ponderous. B.P. quality; Cuxson, Gerrard and Co. Ltd.) is shown in Fig. 1 and 2, the band movement being given as the mean of the leading and following edge movement %  $R_M$ , determined and corrected for variation in column packing as described below.

This reproducible behaviour enables the rate of movement to be controlled over a wide range so that in conjunction with a suitable choice of organic solvent and a sufficiently high initial adsorbent activity most separations can be obtained at a convenient rate. In practice the two adsorbents mentioned, which are of satisfactory grain size for chromatographic use, meet most requirements. Inter-

mediate values of the deactivation tend to give most effective separations as the maximum increase of movement with deactivation generally occurs at low %  $H$  values for weakly adsorbed compounds and at higher values for those more strongly adsorbed. Occasionally the order of the band movement changes with the deactivation, generally at low values of %  $H$  (Fig. 3). Partial deactivation of activated alumina gives a distribution between adsorbent and solvent substantially independent of concentration and bands do not form long trails at one or other edge when this applies.

When the solvent used has affinity for water, equilibrium appears to be established with the deactivated adsorbent and any initial water present in the solvent affects the chromatographic behaviour to some extent. For separations from an aqueous medium this deactivation treatment is ineffective.

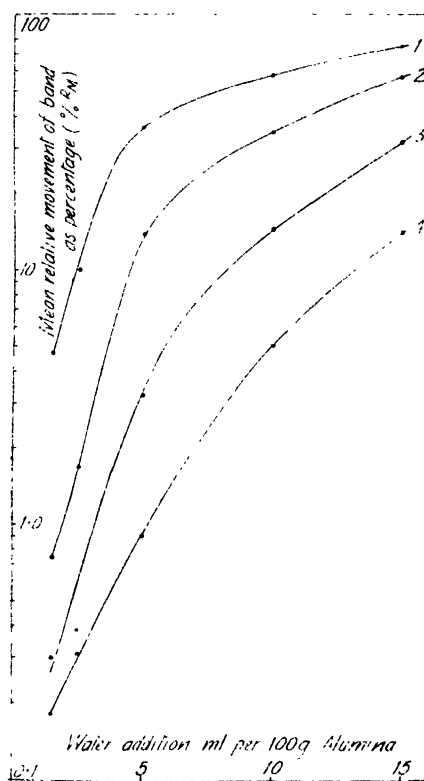


FIG. 4.—Band behaviour of the test mixture in toluene on alumina activated at 330° C for 4 hr.

Curve 1. Red band.  
 „ 2. Blue band.

Curve 3. Purple band.  
 „ 4. Violet band.

Fig. 3 shows the effect of partial deactivation on the distribution constant, calculated from the %  $R_M$  values shown in Fig. 1 by means of eqn. (3). There is a very considerable change in the distribution with practically a linear relation between  $\log K$  and %  $H$  when the deactivation exceeds 2.5 %  $H$ .

**The Effect of Conditions of Activation of Alumina on the Deactivation Behaviour.**—Activated alumina may be prepared<sup>12</sup> by heating aluminium trihydroxide at atmospheric pressure, the product,  $\gamma$  alumina, retaining some water to an extent varying with the activation temperature; it may also contain

<sup>12</sup> Holmes, Lava, Delfs and Cassidy, *J. Biol. Chem.*, 1933, **99**, 417.

alkaline impurities<sup>13</sup> which can if necessary be removed, but these rarely upset the chromatographic behaviour. Satisfactory commercial products are available which obviate the rather troublesome activation. To find the effect of activation conditions on the behaviour after water deactivation, dried hydrate of alumina (British Aluminium Co. Ltd.) was heated in an open pan at temperatures and for periods on temperature given below, together with the moisture content of the products determined by ignition to about 800° C.

Temperature .. °C	250	330	330	330-360	400	500-550
Time .. .. hr.	4	4	8	24	4	4
Moisture content %	11.3	8.8	7.9	5.3	3.9	1.7

Each sample was deactivated with from 1.5 % to 15 % water addition (% H) and

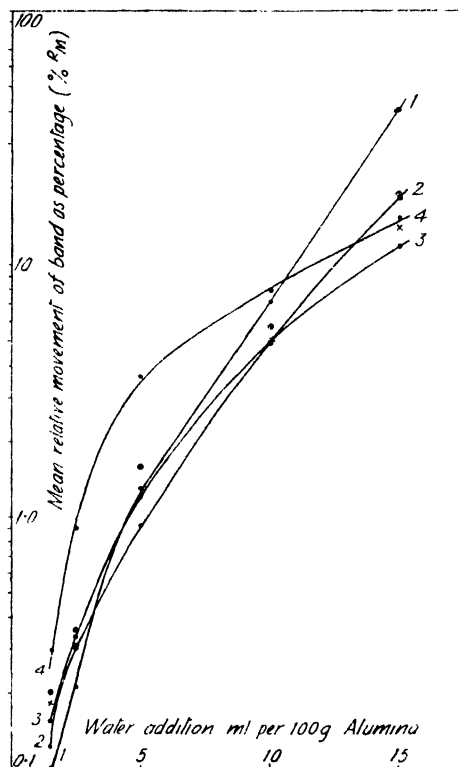


FIG. 5 --Effect of conditions of activation on the relative movement of the violet band.

Curve 1.	Activated at	250° C	4 hr.
" 2.	"	330° C	4 "
" 3.	"	330° C	8 "
" 4.	"	330°-360° C	24 "
" 5.	"	400° C	4 "
" 6.	"	500°-550° C	4 "

band movements measured using a toluene solution of four components which gave distinctive highly coloured bands covering a convenient range of adsorption affinity. The 10 ml. solution added to each column contained

- 0.77 mg. 1-Methylaminoanthraquinone (red, least adsorbed band),
- 0.34 mg. 1 : 4-Dimethylaminoanthraquinone (blue band),
- 1.28 mg. 1-Amino-4-methylaminoanthraquinone (purple band),
- 0.58 mg. 1 : 4-Diaminoanthraquinone (violet, most adsorbed band),

<sup>13</sup> Siewert and Jungnickel, *A.C.S. Abstr.*, 1943, **37**, 5898.

the actual concentration of the components being unimportant provided sufficient is present to give a highly coloured band. Fig. 4 shows the band behaviour of these components on one sample of adsorbent while Fig. 5 shows the effect of conditions of activation on the behaviour of the most strongly adsorbed component, the results for the others being similarly distributed and displaced to an extent indicated in Fig. 4. The curves in Fig. 5 show the effect of activation temperature, the effect of the heating period between 4 and 24 hr. at 330° C being within the experimental error.

The activation conditions employed have considerably less effect on the behaviour than has the subsequent deactivation treatment. There is no direct relation between the initial moisture content and the water added on deactivation, e.g., samples prepared at 250° and 530° C differ by 9.6 % in initial moisture but

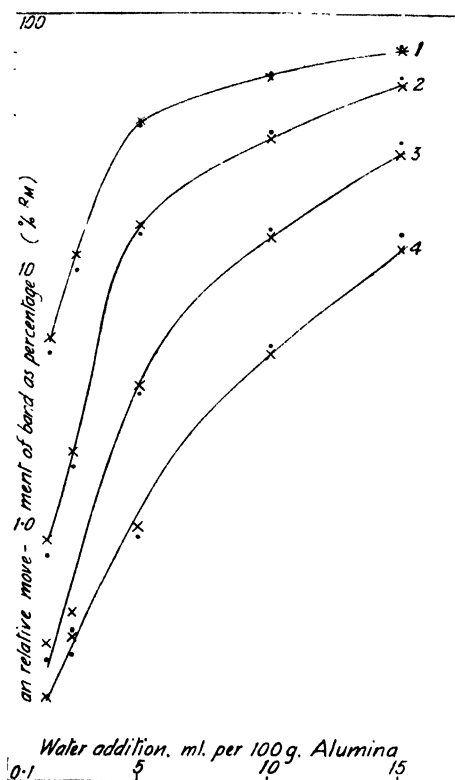


FIG. 6.—Effect of the correction for variation in the ratio of solvent to adsorbent in alumina columns (x) and curves show the uncorrected values; (•) show the corrected values corresponding to the curves shown in Fig. 4. The  $i/m$  values were: 1.5 %  $H$  0.580; 2.5 %  $H$  0.583; 5.0 %  $H$  0.542; 10 %  $H$  0.462; 15 %  $H$  0.425.

give practically the same relative band movement when a further 10 % water is added to both. For low values of %  $H$  the band movement increases with activation temperature, but the extent to which the band movement increases on further addition of water decreases with activation temperature. Qualitative tests show that activation temperatures above 500°–550° C give a progressive decrease in activity. The quality of activated alumina probably depends to some extent on the nature of the starting material and other factors, but the general behaviour on deactivation is similar.

**The Measurement of Band Movement.**—The tubes used were 2 cm. diam. and 55 cm. long having a compact cotton wool retaining plug. Well-packed columns free from air bubbles were obtained from 150 g. alumina or

70 g. magnesium carbonate which were added as a slurry in toluene and settled, with assistance of intermittent tapping, to a constant column length before use; the interstitial solvent was determined by weighing the column before and after adding the adsorbent. The temperature remained close to 20°C throughout and no temperature correction was applied. The solvent used was high-grade technical toluene which had been passed through a column of the same adsorbent so as to minimize any possible effect on the activity. The solution under test was added (10 ml. of 0.1 % conc.) when the solvent level had dropped to the adsorption surface and as soon as this entered the column it was washed in with several small (0.5–1 ml.) lots of solvent before raising the solvent level to about 10 cm. above the adsorbent where it was maintained throughout the measurement by means of a constant level arrangement. Care was taken at all stages not to disturb the adsorbent or allow air to enter the column. Gravity flow was employed and was measured by weight. The position of the band edges and the solvent flow gave satisfactory linear relations against time from which the  $R_L$  and  $R_F$  values were obtained by means of the previously determined length of column occupied by 1 g. of interstitial solvent, and these are given throughout this paper as a percentage of the solvent movement.

A typical column was of 3.303 cm.<sup>2</sup> cross-sectional area and 43.9 cm. length  $L$ , containing 150.3 g. ( $m \times L$ ) activated alumina of 5 %  $H$  and 81.29 g. ( $i \times L$ ) interstitial solvent; the latter occupies 0.540 cm. per g. solvent and for the observed flow of 54.34 g./hr. the linear movement of solvent was 29.29 cm./hr. The solvent movement varied between 23 and 45, average 28.8 cm./hr. for the various columns. The value of  $i/m$  was fairly constant for a single sample of adsorbent but varied from 0.6 to 0.4 as the %  $H$  increased from 1.5 to 15 %, the mean value being close to 0.5. Values of  $R$  corresponding to this mean value of  $i/m$  were obtained from the measurements on alumina columns from

$$R_{\text{corrected}} = \frac{1}{1 + \frac{1}{0.5} \times \frac{i}{m} \frac{(1-R)}{R}}.$$

The magnesium carbonate columns gave a mean value for  $i/m$  of 1.17, which was used in place of 0.5 for their correction.

The effect of the correction for  $i/m$  is shown in Fig. 6 where the uncorrected values corresponding to Fig. 4 are shown by (×) with curves drawn through these points and the corrected values—corresponding to the curves shown in Fig. 4—are indicated by (•). The correction is relatively small and without significant effect on the general relation between band movement and adsorbent activity.

The reproducibility of the band movement measurement was tested in a series of columns using a band having  $R_M$  51 %; the standard deviation for the  $R_L$  and  $R_F$  values was 3.3 and 2.7 and for  $R_M$  2.0.  $R_L/R_M = 1.15$ , with standard deviation 0.06. The behaviour of these columns was found substantially unchanged after use in a series of measurements.

The reproducibility is reasonably satisfactory for comparative purposes involving fairly large differences, but apart from errors due to the difficulty of observing the exact position of the band edge and the occasional inclination of the bands there is a further error arising from the bands and presumably the solvent moving faster in mid-column than at the periphery. Since a uniform solvent movement over the cross-sectional area of the column has been assumed the  $R_M$  values are too low, results given below indicating this error to be about 11 % but no correction has been applied for this factor. Provided the comparisons are carried out in similar columns, as in the present case, this source of error should remain reasonably constant.

**The Relation between Adsorption and Band Movement.**—Preliminary adsorption measurements were made allowing a solvent solution to reach equilibrium with an alumina adsorbent and determining the equilibrium concentration, but this procedure was limited to low values of the latter by the low solubility of the compounds used and the photometric method employed. The results fitted the Freundlich adsorption equation,

$$\log_{10}(\text{specific adsorption}) = \log_{10} K + \alpha \log_{10}(\text{equilibrium concentration}),$$

$\alpha$  being about 0.75 for activated alumina but increasing to 1.0 on partial deactivation with water and becoming slightly greater than 1 for weak adsorption. Within a rather large experimental error there was agreement between the observed  $R_M$  value and that calculated from  $K$  by means of eqn. (2).

To obtain measurements at higher concentrations a 2-cm. column packed with 50 g. partially deactivated Type O alumina and of known interstitial volume was used, the solution being fed through the column until the issuing solution was at the initial concentration and the amount adsorbed determined

TABLE I

THE RELATION BETWEEN THE BAND MOVEMENT CALCULATED FROM ADSORPTION MEASUREMENT AND THE OBSERVED VALUE

Adsorbent: Partially deactivated Type O Activated Alumina.

%  $H$  = ml. water added per 100 g. adsorbent.

(w) measurement for band washing out.

(p) " from peripheral observation.

Adsorbate	Conditions	% $R$ calc. from adsorption	% $R$ obs.		
			$R_L$	$R_F$	$R_M$
1 : 4-Di- <i>p</i> -toluidino-anthraquinone	Toluene : 5 % $H$ Conc. 46 mg. %	73.2 } 72.7 } 73.0	(w) 81.7 (p) 74.8	55.8 51.4	68.7 63.1
		76.0 } 68.0 } 72.0	(p) 75.0	51.6	63.3
1-Methylamino-anthraquinone	Toluene : 5 % $H$ Conc. 115 mg. %	55.0 } 55.1 } 55.0	(p) 57.0	38.0	47.5
		55.9 } 54.1 } 55.0	(p) 57.3	37.0	47.2
		53.5 } 54.6 } 54.0	(p) 56.1	36.6	46.4
1-Amino-2-methyl-anthraquinone	Toluene : 5 % $H$ Conc. 46.2 mg. %	37.0 } 37.2 } 37.1	(w) 54.2 (p) 34.0	20.8 20.2	40.1 31.6
		36.2 } 36.4 } 36.3	(w) 50.3 (p) 33.0	27.4 28.2	38.8 30.6
		34.6 } 36.7 } 35.6	(w) 47.3 (p) 31.2	27.9 29.0	37.6 30.1
1-Amino-4-methyl-aminoanthraquinone	Toluene : 5 % $H$ Conc. 23.1 mg. %	9.1 } 9.5 } 9.3	(w) 13.3 9.8	7.2 7.5	10.3 8.6
	Benzene Conc. 4.0 mg. %				
	5 % $H$	17.7	(p) 22.4	14.1	18.2
	10 % $H$	46.2	(p) 51.0	35.3	43.1
1 : 4-Diaminoanthraquinone	Toluene : 5 % $H$ Conc. 7.5 mg. %	8.8 } 9.2 } 9.0	(p) 7.2	7.0	7.1

by difference and also by washing the band from the column, allowance being made for the solution held by the cotton wool retaining plug. The relative band movement was determined in the same column by observing the peripheral movement and in some cases by observing when the band started and finished washing from the column. The results are summarized in Table I.

There is reasonable agreement between the band movement calculated from adsorption measurements and the observed  $R_M$  values, the values obtained

by washing from the column being 5.1 % high and those obtained from peripheral observations 11.1 % low; as indicated above the latter are likely to be too low due to non-uniformity of solvent flow across the cross-sectional area of the column. The results confirm that band movement on the partially deactivated alumina adsorbent used is independent of concentration. A considerable difference is shown between the band movement of the leading and following edges which in the case of the peripheral results is a measure of the band widening further considered below: the somewhat greater difference obtained when the band is washed out represents the widening between the leading edge in mid-column and the following edge at the periphery.

**The Widening of Bands.**—(i) The leading and following edges of a band move down at a uniform rate and the widening of each edge is given by  $R_L/R_M$ . The widening is not uniform over the whole band but is greater towards the edges than for the main deeply coloured portion, the movement of 1-methyl-aminoanthraquinone in toluene on Type O 5 % H alumina being:

		$R_F$	$R_L$	$R_M$	$R_L/R_M$
Edges of band	..	43.2	59.2	51.2	1.15
Main portion of band	..	45.1	55.7	50.4	1.10

(ii) The  $R_L/R_M$  value is substantially the same for the bands of all components present in a column, independent of the  $R_M$  value and is little affected by the extent of deactivation. Table II gives results from a set of measurements on the activation of alumina using the four component mixture as described above, which are typical of the six samples examined in this connection.

TABLE II

$R_L/R_M$  FOR THE FOUR COMPONENT MIXTURE ON FIVE 2-CM. COLUMNS: ALUMINA ACTIVATED AT 330° C FOR 8 HR. AND PARTIALLY DEACTIVATED

Column Deactivation (% H)	$i/m$	Violet Band	Purple Band	Blue Band	Red Band	Average
$R_L/R_M$						
1.5	.565	1.14	1.04	1.05	1.12	1.09
2.5	.559	1.12	1.06	1.05	1.06	1.07
5	.526	1.29	1.23	1.17	1.11	1.20
10	.439	1.08	1.09	1.04	1.09	1.07
15	.403	1.09	1.07	1.04	1.07	1.07
Average	—	1.14	1.10	1.07	1.09	1.10
$R_M$ %						
1.5	—	.2	.4	1.2	7.9	2.4
2.5	—	.4	.6	2.3	14.1	4.3
5	—	1.6	5.0	19.3	41.2	16.8
10	—	5.6	15.4	38.1	61.1	30.0
15	—	18.7	39.3	64.5	80.6	50.8
Average	—	5.3	12.1	25.1	41.0	20.9

(iii) Similar band widening occurs in the absence of adsorption in passage through a column of 2 cm. diam. packed with non-adsorbent particles of similar grain size. 1:4-Di-*p*-toluidinoanthraquinone is not adsorbed from toluene solution by slightly moist and unactivated dried hydrate of alumina, fairly similar in particle size to the activated product used in Table II. Tests gave  $R_M = 95.1$  % within the anticipated peripheral error of 100 %. The  $R_L/R_M$  value was 1.15. The concentration of the solution leaving the column was also measured photometrically, the results confirming the band widening as shown in Fig. 7.



An increase in the viscosity of the solvent by addition of liquid paraffin reduced the flow rate and band movement to a quarter but did not change the  $R_L/R_M$  value.

(iv) The above results indicate that the band widening is not dependent on adsorption but is directly proportional to the distance of travel along the column, since  $R_L/R_M$  is substantially the same for all the bands in a column,  $\Delta D$  is constant and  $R = \Delta x/\Delta D$ .

(v) The bands from a solution containing several components move through a column in such a way that they have the same width at any given position in the column. Fig. 8 shows the observed band width plotted against the mean band position for the four component mixture referred to above, using a column of 2.4 cm. diam. There is appreciable observational error indicated

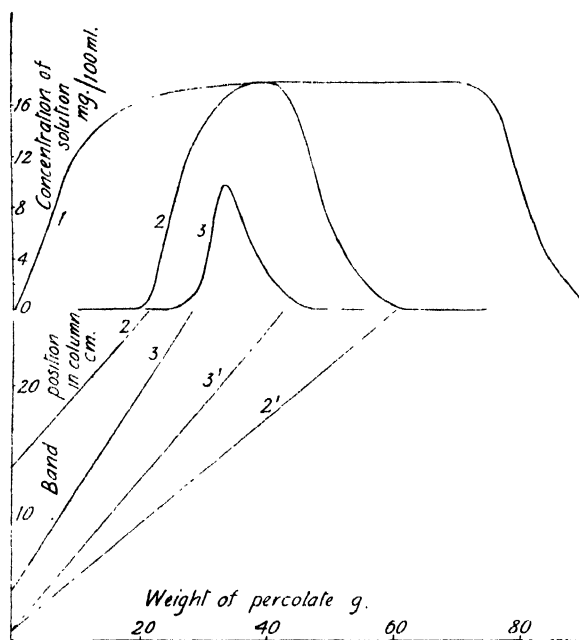


FIG. 7. Behaviour on a non-adsorbent column. Band position and concentration leaving column for 1:4-di-*p*-toluidinoanthraquinone in toluene on a 2-cm. column of unactivated alumina.

Curve 1 40 ml. (34.6 g.) initial solution conc. 17.8 mg. per 100 ml.  
 " 2 20 " (17.3 g.) " " " " "  
 " 3 5 " (4.3 g.) " " " " "

by vertical lines on which the mean value is marked. In the upper part of the column the widening is proportional to the distance travelled but beyond 25 cm. the rate of widening decreases, perhaps due to the band edges becoming too weak to be observed. The width of the four bands practically overlaps, although the leading band passed the 15 cm. position 48 hr. before the last band. A possible explanation of the effect is that the major part of the band width is due to the widening effect, which is similar for all the bands, and small differences arising from the length of the initial bands are concealed by the observational error.

(vi) EFFECT OF SOLVENT FLOW ON BAND WIDENING.—A moderate increase in the solvent movement  $D$  from 0.6 to 3.0 cm./min. had little effect in a 2-cm. column on the  $R_L/R_M$  value at 1.13. Increasing the flow to 15 cm./min. caused an appreciable initial widening of the band after which the rate of widening decreased to give an average value of 1.23.

(vii) EFFECT OF COLUMN DIAMETER.—The band widening appears to decrease as the column diameter increases, columns of 2, 5 and 7.5 cm. diam. giving

(viii) A possible cause of band widening may be that the interstitial capillary spaces are not of uniform size and flow through some outdistancing flow through others leads to dilution and consequent widening of the band.

Although the band movement is faster in mid-column the rate of diffusion in a column is considered to be too small for diffusion from the mid-column to periphery to account for the widening. In addition the diffusion would be affected by the strength of adsorption, the viscosity of solvent and the rate of solvent flow.

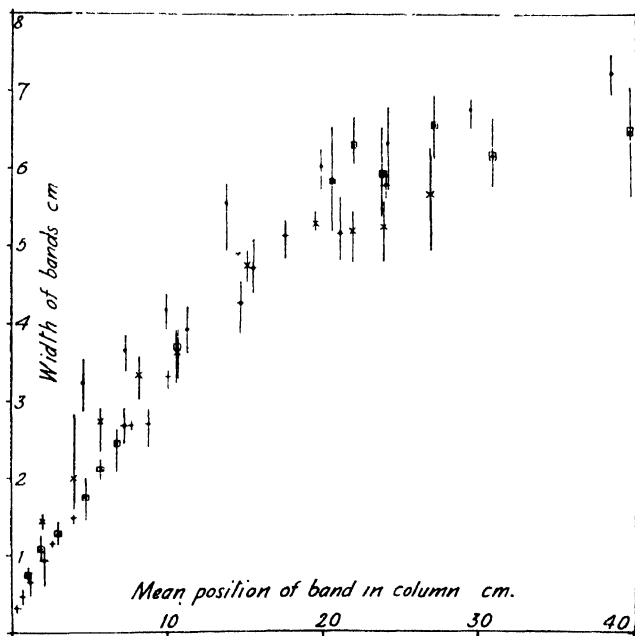


FIG. 8.—Variation in band width with mean position in column.  
Solvent: Toluene; Adsorbent: Alumina activated 300° C 24 hr. 5 % H.  
Column: 2.4 cm. diam.

● Red band.                      □ Purple band.  
◀ Blue band.                  -●- Violet band.

Some diffusion is to be expected when a solution moves along a capillary space between two solvent layers since the movement is a maximum in mid-capillary and zero at the capillary surface. But if this was the full explanation band widening might be expected to be more sensitive to the viscosity of the solvent and rate of solvent movement, and to increase in a more closely packed column.

**The Length of the Initial Band.**—From eqn. (2) the length of band obtained on adding a solution of a pure compound to a column  $x_0$  should equal  $RD_0$ , the product of the relative band movement and the length of column occupied by a volume of interstitial solvent equal to the volume of solution added. Fig. 9 shows the observed  $x_0$  plotted against  $R_L D_0$  and  $R_F D_0$  from the results of 50 measurements covering 20 pure compounds, in which 10 or 20 ml. of solution was used,  $D_0$  being approximately 5 or 10 cm.

There is fairly satisfactory agreement between  $x_0$  and  $R_L D_0$ , whereas the value of  $R_F D_0$  is only about 70 % of  $x_0$ . For strongly adsorbed compounds the initial band is very small and difficult to measure and the  $x_0$  value tends to be too

large in these cases. The average values for the 40 measurements in which the band exceeded 1 cm. length were :

$$\begin{array}{lll} x_0, 4.59 \text{ cm.}; & R_L D_0, 4.66 \text{ cm.} & R_F D_0, 3.40 \text{ cm.} \\ & R_L D_0/x_0, 1.00, \text{ std. dev. } 0.17 & R_F D_0/x_0, 0.71, \text{ std. dev. } 0.14 \end{array}$$

The agreement between  $x_0$  and  $R_L D_0$  is to be expected since the initial band is itself a leading edge and its length will be correspondingly greater than the theoretical  $R_M D_0$  and equal  $R_L D_0$ .

**The Relation between Chemical Constitution and Chromatographic Behaviour of Anthraquinone Compounds.**—Strain<sup>14</sup> has shown that the relation between constitution and chromatographic behaviour frequently depends on the solvent and adsorbent conditions used and this is found to apply to

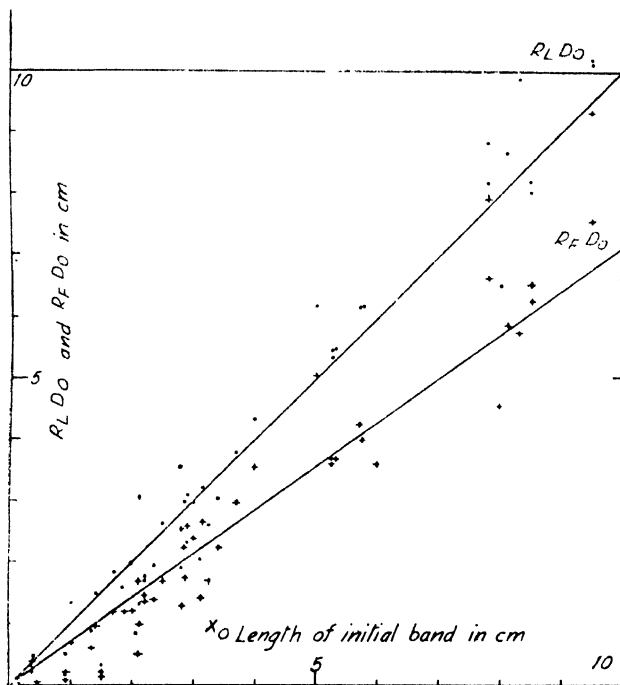


FIG. 9.—Relation between  $x_0$  and  $R_L D_0$  (●) or  $R_F D_0$  (+).

anthraquinone compounds, in some cases a change in the activity of the adsorbent by water addition being sufficient to alter the order in which bands move. Within this qualification certain general rules serve as a guide to the behaviour and appear to be governed by two factors: (i) the specific action of certain groups such as hydroxy and amino in increasing the adsorption, probably as Meunier and Vinet<sup>15</sup> suggest due to hydrogen bonding between the group and the adsorbent; (ii) the avidity of the various compounds for the solvent employed acting against a fairly general adsorptive power.

The order of increasing adsorption for mono-substitution is generally halogeno, nitro, arylamino, alkylamino, amino, acylamido, hydroxy group in side chain, and hydroxy group attached to nucleus. There does not appear to be any systematic relation between mono-substitution in the 1 and 2 positions.

Adsorption generally increases with increasing number of substituent groups of the same composition, to an extent which varies with the position occupied

<sup>14</sup> Strain, *Ind. Eng. Chem. (Anal.)*, 1946, **18**, 605.

<sup>15</sup> Meunier and Vinet, *Chromatographie et Mesomerie* (Masson & Cie, Paris).

and the nature of the substituent, but decreased adsorption from aqueous medium occurs in the case of the sulphonate group. The introduction of further groups of different composition may either increase or decrease the adsorption, thus methyl, halogeno and arylamino groups tend to decrease and amino, methoxy and hydroxy to increase the adsorption.

### Discussion

The simple method described for the deactivation of adsorbents gives remarkable control over the chromatographic behaviour and is easily applied as a further variable to the choice of solvent and adsorbent when selecting the conditions for any particular separation. It enables the separations to be obtained at a convenient rate and in this connection is of considerable assistance when a flowing chromatogram is combined with photometric measurement for the quantitative determination of the components.<sup>16 17</sup>

Appreciable heat of wetting is generated when a solvent is added to an adsorbent, even after partial deactivation and as Müller<sup>3</sup> has shown, the heat generated gives a measure of the activity. It would appear that the adsorption of a solute depends on competition between the solute, water and solvent for the available active surface. In the case of activated alumina a small degree of deactivation renders the distribution of a solute between solvent and adsorbent independent of the concentration, a factor which facilitates the chromatographic separation; under these conditions fairly satisfactory agreement has been obtained between the mean relative movement of the bands, the length of the initial band and the values predicted from theory.

The solvent flow is greater in mid-column than at the periphery where the almost spherical adsorbent particles are in contact with a surface of much smaller curvature, and the packing would be expected to be less perfect. Although the chromatograph columns of alumina show dilatancy the particles are not in closest packing—the interstitial volume is too high for this—and it seems likely that they are in a flocculated state similar to that described by Kruyt and Selms<sup>18</sup> for suspensions of silica particles in organic solvent, in which case the glass surface of the tube may be linked to the adsorbent particles by forces similar to those responsible for the flocculation. The troublesome band widening appears to be related to the manner of flow through the interstitial spaces.

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<sup>16</sup> Cropper and Strafford, *J. Soc. Chem. Ind.*, 1944, **63**, 268.

<sup>17</sup> Cropper, *Analyst*, 1946, **71**, 263.

<sup>18</sup> Kruyt and Selms, *Rec. trav. chim.*, 1943, **62**, 407.

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## EQUILIBRIUM AND RATE STUDIES OF CATION-EXCHANGE WITH MONOFUNCTIONAL RESINS

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The preparation of sulphonated cross-linked polystyrene and of cross-linked polymethacrylic acid is described. The sodium-hydrogen exchange equilibria for both materials have been examined. The former resin is shown to behave as a monofunctional strong acid and the latter as a monofunctional weak acid. The application of the law of mass action to the exchange equilibria is discussed.

A study of the rate of sodium-hydrogen exchange with sulphonated cross-linked polystyrene suggests that at low concentrations of sodium ions in solution the rate-determining mechanism is diffusion of ions through a thin film of liquid surrounding the resin particle. The influence of hydroxyl ion concentration on the rate of exchange of sodium for hydrogen with both resins is described, and the conditions under which the diffusion of ions within the resin particle may become the rate-controlling process are discussed.

In the application of ion-exchange resins to chromatographic techniques, more information is required on the fundamental molecular and ionic processes involved. Correlation of such information with the basic chemical and macromolecular structure may be expected to lead to the development of improved materials. In equilibrium and rate studies, monofunctional resins obtained by addition polymerization, e.g., sulphonated cross-linked polystyrene and cross-linked polymethacrylic acid, offer many advantages. In contrast to earlier materials obtained by polycondensation, e.g., sulphonated phenol-formaldehyde resins, these materials possess a fairly well-defined structure which may be systematically varied. They may also be examined over a wide range of pH without complicating factors arising due to the presence of different types of ionizable group. Moreover, the technique of suspension polymerization enables the resins to be prepared in the form of spherical beads which are especially suitable for rate studies.

In this paper the preliminary results of an investigation into the equilibrium and rate processes with sulphonated cross-linked polystyrene and cross-linked polymethacrylic acid are presented.

### Experimental

**Preparation of Cation-exchange Resins.**—SULPHONATED CROSS-LINKED POLYSTYRENE. This material was prepared by the sulphonation of a cross-linked polystyrene bead polymer as described by D'Alelio.<sup>1</sup> Styrene was co-polymerized at 80° C for 18 hr. with *ca.* 10 % divinylbenzene, 1 % benzoyl peroxide being employed as catalyst. The co-polymer was sulphonated with concentrated sulphuric acid at 100° C for 8 hr. using 1 % silver sulphate as catalyst. The maximum capacity of the product (5.25 milli-equivalents of base per g. dry hydrogen form) was independent of particle size and agreed with the value calculated for a monosulphonic acid. The material was hygroscopic and the dry hydrogen form absorbed approximately 80 % water at 20° C. On conversion from the wet hydrogen form to the wet sodium form a decrease in volume of 6 %–7 % was observed.

CROSS-LINKED POLYMETHACRYLIC ACID. The carboxylic type exchange resin was prepared from methacrylic acid and divinylbenzene. Commercial methacrylic acid redistilled *in vacuo* was polymerized with *ca.* 10 % divinylbenzene in the presence of 1 % benzoyl peroxide. Polymerization was carried out in a sealed tube at 60° C for 24 hr. The product was treated with 2 N NaOH to remove soluble material, washed and dried. The maximum capacity (9.2 milli-equivalents of base per g. dry hydrogen form) was consistent with the value calculated from the composition of the monomer mixture. The dry material absorbed approximately 130 % of water at 20° C and an increase in volume of approximately 75 % was observed on conversion from the wet hydrogen to the wet sodium form.

After preliminary cycling in a column between 2 M NaCl or 2 N NaOH and 2 N HCl, the cation-exchange resins were converted to the hydrogen form and washed with distilled water. Washing was continued until the pH of the effluent attained a value of 4.0 or higher. If necessary, fines were removed by elutriation and the ion-exchange resins were air-dried to a uniform moisture content.

<sup>1</sup> D'Alelio, U.S. Pat. 2,366,007.

**Equilibrium Studies.**—Samples of the cation-exchange resins in the hydrogen form were weighed out into a series of bottles and further samples were taken for moisture content determinations. The latter were dried to constant weight over  $P_2O_5$  in a vacuum desiccator. Different amounts of NaOH solution with or without NaCl solution were added to the samples of resin and the solutions made up to 50 ml. For the sulphonated cross-linked polystyrene the liquid-solid ratio was 100/1, for the cross-linked polymethacrylic acid 200/1. The solutions were allowed to stand with occasional shaking until equilibrium was attained (1–7 days). Aliquot samples of the solutions were then withdrawn and titrated with standard HCl or NaOH to determine the extent of exchange. The pH values of the solutions were determined using a Cambridge pH meter and glass electrode. For the measurement of pH values greater than 9.0, a Cambridge Alki electrode was employed.

**Rate Studies.**—To obtain samples of resin of approximately uniform particle diameter, the resins were sieved in the air-dry state using calibrated B.S. sieves. For the determination of rates of exchange two methods were employed.

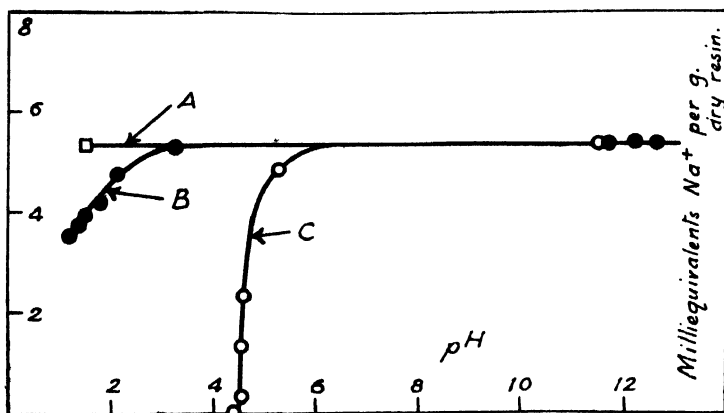


FIG. 1.—Sulphonated cross-linked polystyrene. Relationship between  $Na^+$  ion taken up by the resin and pH.

- A. In presence of 5 M NaCl. B. In presence of 0.1 M NaCl. C. In absence of NaCl.

(a) **INDICATOR METHOD.** This method, which is only applicable to sulphonated cross-linked polystyrene, depends on the fact that whether the solution is acid or alkaline, exchange will proceed virtually to completion if the ratio  $[Na^+]/[H^+]$  in solution is sufficiently high (see below). The hydrogen form of the resin is stirred with a solution of NaCl and NaOH, the latter being less than sufficient to neutralize the hydrogen ions liberated in the exchange process. The solution, initially alkaline, becomes acid when the amount of exchange just exceeds the amount of alkali originally added. An indicator preferably of the anionic type is used to show this change.

A weighed amount of resin of known moisture content was added to a known volume of water containing a few drops of bromo-cresol green indicator solution (0.04 % solution in water) in a small beaker. The mixture was stirred vigorously with a magnetic stirrer and a suitable mixture of NaCl and NaOH solutions added. The time elapsing between the addition of the alkali solution and the colour transition (blue→yellow) was measured with a stop-watch. All experiments were carried out at room temperature (18°–22° C) and with a constant volume of solution.

(b) **SHALLOW-BED METHOD.** A simple modification of the method used by Boyd, Adamson and Myers<sup>2</sup> was employed. The resin sample (ca. 0.1 g.) was supported

<sup>2</sup> Boyd, Adamson and Myers, *J. Amer. Chem. Soc.*, 1947, **69**, 2836.

on stainless steel gauze or a sintered-glass disc. The resin was first converted to the hydrogen form and washed free of acid. The solution containing sodium ions was then passed through the bed for an appropriate time at a known flow-rate. The bed was then immediately washed with a stream of distilled water. In the case of the sulphonated cross-linked polystyrene, the amount of residual hydrogen ion was determined by displacement, using an excess of NaCl solution and titrating the solution with standard alkali. For the cross-linked polymethacrylic acid, the amount of exchange was determined by removing the sodium ion with a measured volume of standard acid and back titration of the acid solution.

### Results

**Exchange Equilibria.**—The amount of sodium ion taken up at equilibrium by the resins and its dependence on pH and on the ratio  $[Na^+]/[H^+]$  in solution is shown in Fig. 1, 2 and 3.

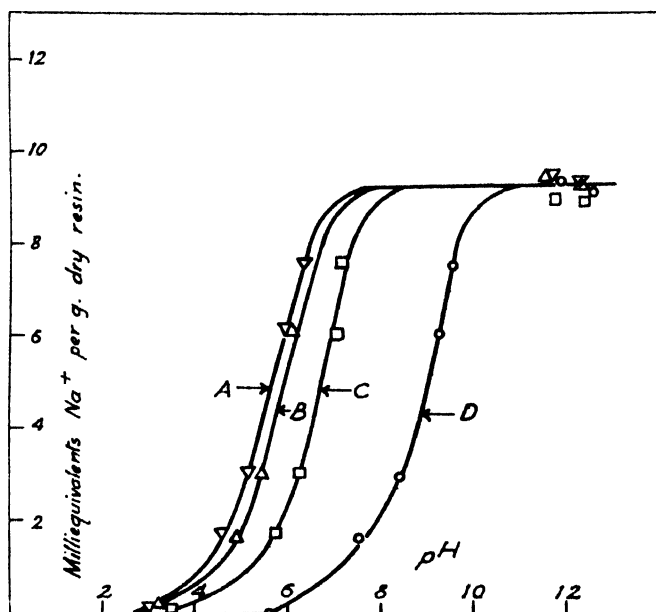


FIG. 2.—Cross-linked polymethacrylic acid. Relationship between  $Na^+$  ion taken up by the resin and pH.

- A. In presence of 2 M NaCl. B. In presence of 1 M NaCl. C. In presence of 0.1 M NaCl. D. In absence of NaCl.

It will be seen from Fig. 3 that the  $Na^+$  ion taken up by both resins was dependent only on  $[Na^+]/[H^+]$  in solution and not on  $[Na^+]$  or  $[H^+]$  separately. In the case of the sulphonic acid type exchanger, provided the  $[Na^+]/[H^+]$  ratio in solution is greater than 100/1, virtually complete replacement of hydrogen by sodium ion is effected. For the exchange resin containing carboxylic groups, a  $[Na^+]/[H^+]$  ratio of at least 10<sup>6</sup>/1 is necessary to effect complete conversion to the sodium form.

**Exchange Kinetics.**—Using the indicator method it was found that above a minimum rate of stirring the results obtained were independent of the stirring rate. This was found to apply over the whole range of sodium ion concentrations studied. Under the conditions employed, however, the degree of mixing of resin and solution may be expected to be lower than that attained with the shallow-bed method.

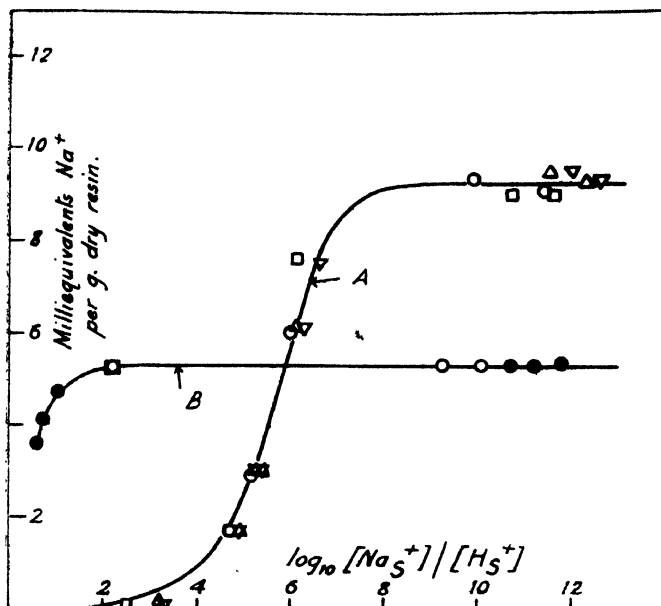


FIG. 3.—Relationship between Na<sup>+</sup> ion taken up by the resin and  $\log_{10} [Na_S^+]/[H_S^+]$ .  
A. Cross-linked polymethacrylic acid. B. Sulphonated cross-linked polystyrene.

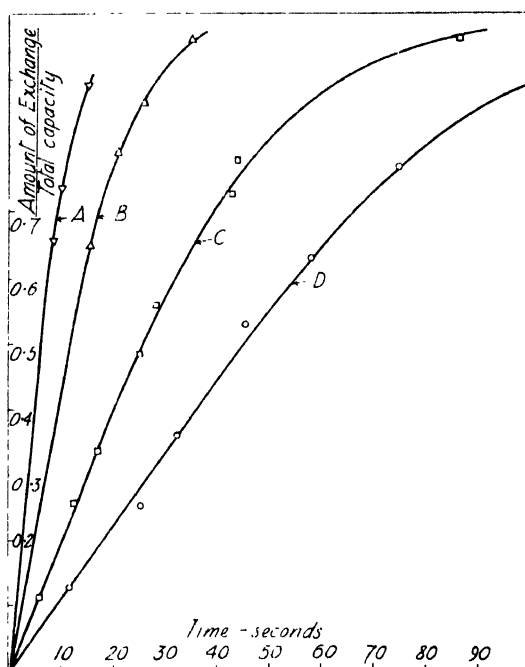


FIG. 4.—Sulphonated cross-linked polystyrene. Exchange kinetics at low Na<sup>+</sup> ion concentrations. (Indicator method.)

A.	Air-dry particle diameter	50-100 $\mu$	$[Na_S^+]$ 0.048-0.050 M
B.	"	"	$[Na_S^+]$ 0.026-0.029 M
C.	"	"	$[Na_S^+]$ 0.045-0.050 M
D.	"	"	$[Na_S^+]$ 0.023-0.028 M



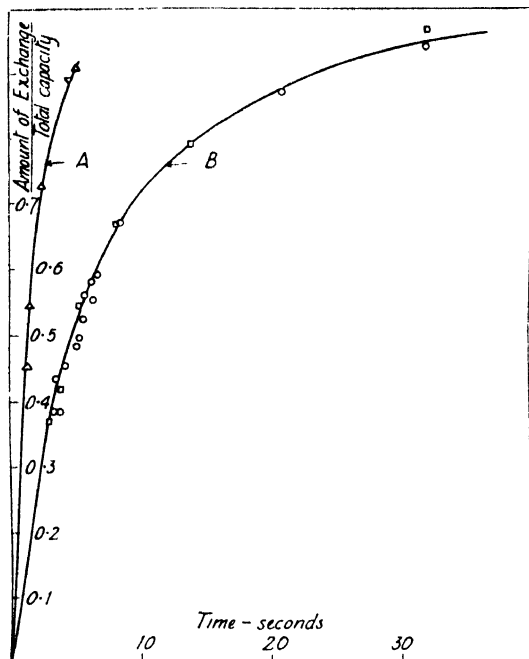


FIG. 5.—Sulphonated cross-linked polystyrene. Exchange kinetics at high  $\text{Na}^+$  ion concentrations. (Indicator method.)

A. Air-dry particle diameter 50–100 $\mu$	$\nabla [\text{Na}_s^+]$ 2.18 M
	$\Delta [\text{Na}_s^+]$ 1.09 M
B. Air-dry particle diameter 300–400 $\mu$	$\square [\text{Na}_s^+]$ 2.18 M
	$\circ [\text{Na}_s^+]$ 1.09 M

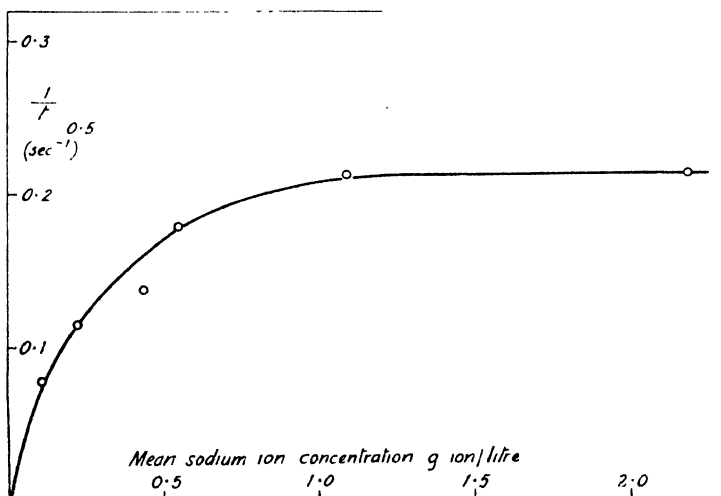


FIG. 6.—Sulphonated cross-linked polystyrene. Relationship between half-life and  $\text{Na}^+$  ion concentration. (Indicator method.) Air-dry particle diameter 300–400  $\mu$ .

The effect of sodium ion concentration in solution and the particle size of the resin were examined using the indicator method. The results obtained are shown in Fig. 4 and 5.

At low sodium ion concentrations, as shown in Fig. 4, the exchange proceeds initially at an approximately constant rate but then slows down progressively as the exchange proceeds. At high sodium ion concentrations (Fig. 5) the form of curve obtained is similar but owing to the high rate of exchange under these conditions the initial rate of exchange cannot be determined accurately by the present method. The reciprocal of the time for half-conversion of the resin to the sodium form is plotted against the mean sodium ion concentration in solution in Fig. 6.

It appears that at high sodium ion concentrations the rate of exchange is independent of the sodium ion concentration, whilst at low sodium ion concentrations the rate is proportional to the sodium ion concentration. This is shown also in Fig. 7 where the initial rate of exchange has been plotted against the mean sodium ion concentration in solution.

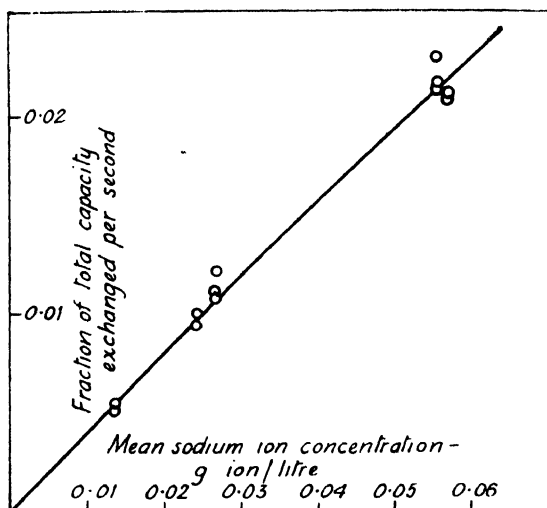


FIG. 7.—Sulphonated cross-linked polystyrene. Relationship between rate of exchange and  $\text{Na}^+$  ion concentration at low  $\text{Na}^+$  ion concentrations. (Indicator method.) Air-dry particle diameter 300–400  $\mu$ .

At low sodium ion concentrations there is a change in the sodium ion concentration as the reaction proceeds. It has however been found that the results are in agreement with the relationship

$$d(\text{Na})/dt = Kw[\text{Na}^+],$$

where  $(\text{Na})$  is the total amount of exchange,  $w$  the weight of resin,  $[\text{Na}^+]$  the sodium ion concentration in solution and  $K$  a constant.

It will be seen from Fig. 4 that at low sodium ion concentrations the rate of exchange with particles of diameter 50–100  $\mu$  is about four times as great as that with particles of diameter 300–400  $\mu$ . Thus, at low sodium ion concentrations the rate of exchange is approximately inversely proportional to the particle diameter. At high sodium ion concentrations no quantitative conclusions can be drawn from the present data, but it is apparent from Fig. 5 that exchange takes place more rapidly with the smaller particles.

The indicator method cannot be used for the direct investigation of the effect of hydroxyl ion concentration on the rate of exchange, and the shallow-bed method was employed for this purpose (see Fig. 8).

Results obtained by the indicator method were confirmed in that, with  $\text{NaCl}$  solutions, the exchange rate was independent of sodium ion concentration

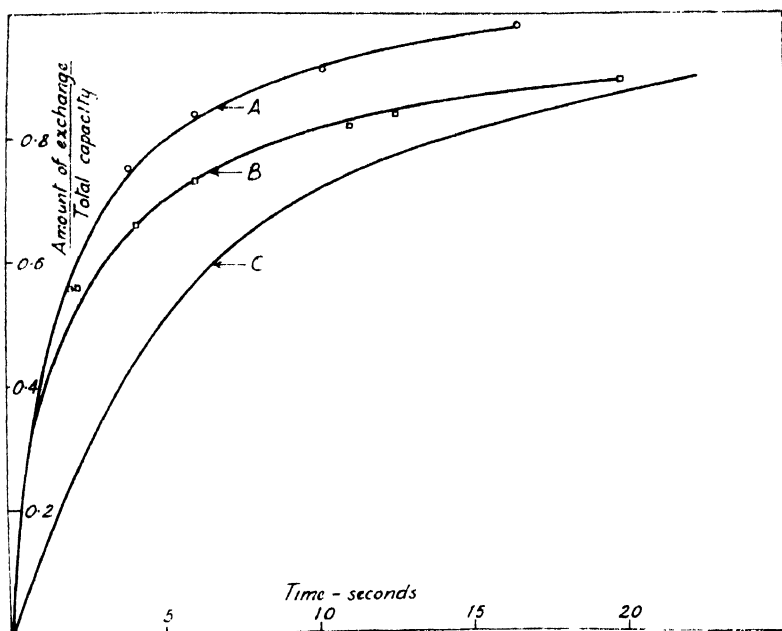


FIG. 8.—Sulphonated cross-linked polystyrene. Exchange kinetics at high  $\text{Na}^+$  ion concentration. Air-dry particle diameter  $300\text{--}400\ \mu$ .

- A. Shallow-bed method. 2 N NaOH and 1 N NaOH solutions. Flow-rates 15 cm./sec. and 30 cm./sec.
- B. Shallow-bed method. 2 M NaCl and 1 M NaCl solutions. Flow-rates 15 cm./sec. and 30 cm./sec.
- C. Indicator method.  $\text{Na}^+$  ion concentration 2 M and 1 M.

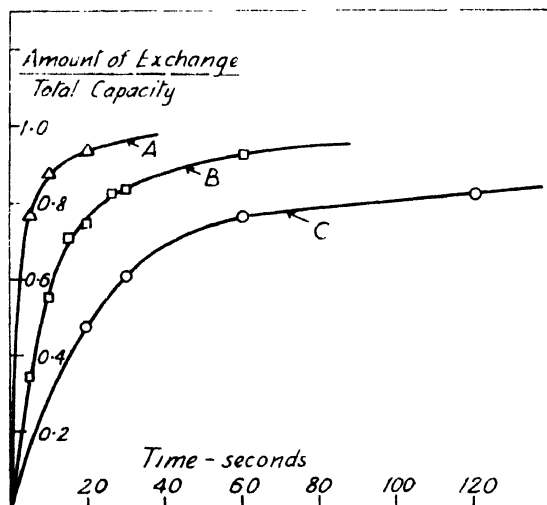


FIG. 9.—Cross-linked polymethacrylic acid. Exchange kinetics at high  $\text{Na}^+$  concentrations. Air-dry particle diameter  $250\text{--}380\ \mu$ . Flow-rate 1 cm./sec.

- A.  $\text{Na}^+$  concentration 2.2 M,  $\text{OH}^-$  concentration 0.2 M.
- B. " " 2.1 M, " 0.1 M.
- C. " " 2.0 M, " 0.025 M.

above 1 M. In addition, it was shown that the rate of exchange increased with increase in hydroxyl ion concentration and was independent of hydroxyl ion concentration above 1 M. Variations in flow-rate from 15 cm./sec. to 30 cm./sec. did not affect the rate of exchange, but the shallow-bed technique using M NaCl gave faster rates of exchange than the indicator method.

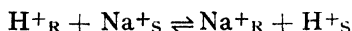
The results obtained with cross-linked polymethacrylic acid, using the shallow-bed method and keeping the flow-rate and  $\text{Na}^+$  concentration virtually constant, are given in Fig. 9.

It will be seen that increase in hydroxyl ion concentration markedly increases the rate of exchange.

### Discussion

**Exchange Equilibria.**—From the study of the exchange equilibria with sulphonated cross-linked polystyrene, we conclude that if the  $[\text{Na}^+]/[\text{H}^+]$  ratio in solution is greater than 100 the resin is fully ionized. It is not possible to say, on the present evidence, whether the resin is fully ionized at all values of  $[\text{Na}^+]/[\text{H}^+]$ . However, it is clear that the resin behaves as a fairly strong acid and only one type of grouping appears to be present.

Consideration of the exchange equilibria of the polymethacrylic acid resin



leads to the mass-action relation

$$\frac{[\text{Na}^+_{\text{R}}]}{[\text{H}^+_{\text{R}}]} = K_1 \frac{[\text{Na}^+_{\text{S}}]}{[\text{H}^+_{\text{S}}]}, \quad (1)$$

activity coefficients being neglected.

$[\text{Na}^+_{\text{R}}]$  and  $[\text{H}^+_{\text{R}}]$  are the concentrations of  $\text{Na}^+$  ion and  $\text{H}^+$  ion in the resin in g. equiv./l.

$[\text{Na}^+_{\text{S}}]$  and  $[\text{H}^+_{\text{S}}]$  are the concentrations of  $\text{Na}^+$  ion and  $\text{H}^+$  ion in the solution in g. equiv./l.  $K_1$  is the relative affinity constant of sodium and hydrogen ions for the resin. By analogy with sulphonic acid resins, we may expect  $K_1$  to have a value of from 1 to 2.

In order to compare eqn. (1) with experimental data, we must have a relationship between  $[\text{H}^+_{\text{R}}]$  and the total concentration of carboxylic hydrogen on the resin. We may assume that

$$\frac{[\text{H}^+_{\text{R}}]}{[\text{Total carboxylic hydrogen}]} = K, \quad (2)$$

where  $K$  is a constant. This leads to the equation

$$\frac{[\text{Na}^+_{\text{R}}]}{[\text{Total carboxylic hydrogen}]} = KK_1 \frac{[\text{Na}^+_{\text{S}}]}{[\text{H}^+_{\text{S}}]}. \quad (3)$$

It has been found that an equation of this type fits the experimental data quite well, with a value of  $2.24 \times 10^{-6}$  for  $KK_1$ .

Alternatively, the ionization of the resin may be assumed to follow the laws holding for dilute solutions. We then write

$$\frac{[\text{H}^+_{\text{R}}][\text{R}^-]}{[\text{HR}]} = K_2, \quad (4)$$

where  $[\text{HR}]$  and  $[\text{R}^-]$  are the concentrations of unionized and ionized resin in g. equiv./l.  $K_2$  may be expected to have a value of the same order ( $10^{-8}$  g. equiv./l.) as for the carboxylic group in simple compounds.

From eqn. (1) and (4) and assuming electro-neutrality of the resin phase, the following relation may be derived:

$$[\text{Na}^+_{\text{R}}] = \frac{-xK_1K_2}{2} + \frac{1}{2} \left[ xK_1K_2 \left\{ xK_1K_2 + \frac{4c}{a \left( 1 + \frac{1}{xK_1} \right)} \right\}^{\frac{1}{2}} \right], \quad (5)$$

where  $x = [\text{Na}^+_{\text{s}}]/[\text{H}^+_{\text{s}}]$ .

$c$  = total capacity of resin in g. equiv. per g. dry resin.

$a$  = volume of wet resin in l./g. dry resin.

It is assumed that  $a$  is constant and independent of both  $[\text{Na}^+_{\text{s}}]$  and  $[\text{H}^+_{\text{s}}]$ . While large variations in the volume of the resin are observed, the effect is small considered in relation to the enormous variations in  $[\text{Na}^+_{\text{s}}]/[\text{H}^+_{\text{s}}]$ .

Since  $K_1 \simeq 1$  and  $[\text{Na}^+_{\text{R}}]$  only becomes significant for values of  $[\text{Na}^+_{\text{s}}]/[\text{H}^+_{\text{s}}]$  greater than  $10^3$ ,  $1/K_1 x$  is less than  $10^{-3}$  and may be neglected in comparison with unity. Therefore

$$[\text{Na}^+_{\text{R}}] = \frac{-xK_1K_2}{2} + \frac{1}{2} \left[ xK_1K_2 \left\{ xK_1K_2 + \frac{4c}{a} \right\} \right]^{\frac{1}{2}} \quad (6)$$

Expressing the amount of sodium ion on the resin in g. equiv. per g. dry resin, we have

$$y = a [\text{Na}^+_{\text{R}}] = \frac{-x(aK_1K_2)}{2} + \frac{1}{2} \left[ x(aK_1K_2) \left\{ x(aK_1K_2) + 4c \right\} \right]^{\frac{1}{2}} \quad (7)$$

From eqn. (5) we see that the amount of sodium taken up by the resin depends on the ratio  $[\text{Na}^+_{\text{s}}]/[\text{H}^+_{\text{s}}]$  and not on  $[\text{Na}^+_{\text{s}}]$  and  $[\text{H}^+_{\text{s}}]$  separately. As constants, eqn. (7) contains only the capacity  $c$  and the parameter  $aK_1K_2$ .

This latter may be estimated.  $K_1 \simeq 1$ ,  $K_2 \simeq 10^{-5}$  g. equiv./l. and since the density of the wet resin is approximately unity,  $a \simeq 10^{-3}$  l./g. Hence  $aK_1K_2 \simeq 10^{-8}$  g. equiv./g. dry resin. Alternatively, a value for  $aK_1K_2$  may be found from the experimental data. It may be shown from eqn. (7) that when  $y = \frac{1}{2}c$ ,

$$x = \frac{1}{2} \frac{c}{(aK_1K_2)}$$

This leads to a value of  $1.05 \times 10^{-8}$  g. equiv./g. for  $aK_1K_2$ .

With  $c = 9.24 \times 10^{-3}$  g. equiv./l. and  $aK_1K_2 = 10^{-8}$  g. equiv./l., values of  $y$  were calculated for various values of  $\log_{10} [\text{Na}^+_{\text{s}}]/[\text{H}^+_{\text{s}}]$  using eqn. (7). The results are represented by the smooth curve in Fig. 3. The agreement between the calculated curve and the experimental points is good in view of the assumptions made.

It is concluded that the behaviour of cross-linked polymethacrylic acid is well-accounted for by assuming that only a small fraction of the carboxylic hydrogen is ionized. The exact relationships governing the degree of ionization is a matter for further investigation.

**Exchange Kinetics.**—Boyd, Adamson and Myers<sup>2</sup> have studied the rates of exchange of various cations on the phenolic resin, Amberlite IR-1. In their discussion they consider three mechanisms, each of which might be rate-controlling under appropriate conditions:

- (i) Diffusion of ions through a thin film of liquid surrounding the particles (or through liquid in macropores inside the particles).
- (ii) Diffusion of ions through the resin material itself.
- (iii) The chemical process of exchange.

In the present work, if the chemical process of exchange were the sole rate-controlling process, the measured rate of exchange would be independent of the particle size. The fact that, both at high and low sodium ion concentrations, the rate varied markedly with particle size would seem to rule out the chemical process as a major rate-controlling factor.

If any type of film diffusion is a rate-controlling process, this is because, despite stirring, rapid flow or any other attempt to make the solution

homogeneous right up to the surface of the particle, there is a thin film inside which mixing is imperfect. (The film may also be due to cracks or macropores in the particle; in this case, the rate of stirring will have practically no effect on the characteristics of the film.)

If the diffusion of sodium ions across the film towards the resin is the sole rate-controlling process, the concentration of sodium ions at the inside boundary of the film will be virtually zero (owing to the diffusion of ions into the particle being very rapid in comparison). Hence the concentration gradient (and the diffusion rate) of sodium ions across the film will be proportional to the concentration of sodium ions in solution. To a first approximation, the film thickness may be assumed to be independent of particle size. The rate of exchange will then be inversely proportional to the particle diameter (provided macropores play a negligible part in film diffusion) since the surface area per particle is proportional to the square of the diameter and the capacity is proportional to the cube of the diameter.

It is probable that the diffusion rates of sodium and hydrogen ions within the resin particle are largely coupled, owing to the powerful electrostatic forces which oppose the entry of anions and also any variation of the total cation concentration in the resin. We may therefore speak of diffusion of ions within the resin being the rate-controlling process without specifying sodium or hydrogen ions separately. If diffusion of ions within the resin is the sole rate-controlling process, this means that the effect of film diffusion has been eliminated and the surface of the resin is in equilibrium with the bulk of the solution. Since in all the present work with sulphonated polystyrene, the  $[Na^+]/[H^+]$  ratio in the solution was at least  $10^4$ , the surface of the resin will be saturated with sodium ions. Hence diffusion of sodium ions into the interior of the resin particle will occur from a constant surface concentration which is independent of the sodium ion concentration in the solution. Hence the rate of exchange will be independent of the sodium ion concentration in the solution.

If diffusion of hydrogen ions through the film away from the resin is the sole rate-controlling process, the rate of exchange will be independent of sodium ion concentration but will be dependent on the hydroxyl ion concentration of the solution. Hydroxyl ions will diffuse towards the surface of the particle and will neutralize hydrogen ions in the film thus increasing the rate of removal of hydrogen ions.

The results obtained at low sodium ion concentrations ( $< 0.1$  M) show exactly the characteristics expected if film diffusion of the sodium ions is the rate-controlling process. At high sodium ion concentrations, the rate is independent of sodium ion concentration and hence the rate-controlling process might be either the diffusion of ions within the particle or the diffusion of hydrogen ions across the film or both. However, Fig. 8 shows that, using the shallow-bed technique, increase in the hydroxyl ion concentration from zero to 1 M increased the exchange rate while a further increase to 2 M had no effect. Hence we may conclude that with M or 2 M NaCl solutions the film diffusion of hydrogen ions is rate controlling, while with N or 2 N NaOH solutions diffusion of ions within the particle is the sole rate-controlling process. The diffusion coefficients of sodium, hydrogen and hydroxyl ions are of the same order. Similar concentrations (1 M) of hydroxyl or sodium ions in solution are therefore likely to be required to eliminate film diffusion of hydrogen and sodium ions as rate-controlling processes.

No satisfactory explanation can be given for the observation that with the shallow-bed method increase in the flow-rate from 15 to 30 cm./sec. failed to increase the rate of exchange with NaCl solutions. The fact that at low sodium ion concentrations the rate was inversely proportional to particle diameter appears to preclude the possibility of macropores playing

an appreciable part in film diffusion. We can only suppose that owing to the highly turbulent nature of the flow in our experiments the effective film thickness was not altered by the apparent flow-rate.

We consider now in more detail the way in which, at high  $\text{Na}^+$  concentrations and virtually zero  $\text{OH}^-$  concentrations, the rate of diffusion of hydrogen ions through the film controls the exchange process. This rate of diffusion is initially zero but increases progressively as the concentration of hydrogen ions outside the particle surface builds up. However, long before the rate of hydrogen ion film diffusion becomes equal to the rate of diffusion of ions within the resin, the ratio  $[\text{Na}^+]/[\text{H}^+]$  in the solution immediately outside the particle surface will have fallen appreciably, causing a decrease in the concentration of  $\text{Na}^+$  ions *inside* the particle surface. Hence the rate of diffusion of ions within the particle will decrease.

When we consider the rate of  $\text{Na-H}$  exchange with the polymethacrylic acid resin, it is apparent that the film diffusion of hydrogen ions will be much more important than with sulphonated polystyrene resins, owing to the much larger  $[\text{Na}^+]/[\text{H}^+]$  ratio ( $10^8$ ) in the solution necessary to maintain the surface of the resin particle saturated with sodium ions. The results (Fig. 9) confirm this expectation.

In conclusion, it appears that for the diffusion of ions within the resin to be the rate-controlling process, it is necessary to have in solution high concentrations of both  $\text{Na}^+$  and  $\text{OH}^-$  ions.

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## CATION EXCHANGE WITH A SYNTHETIC PHENOLSULPHONATE RESIN

### Part V. Kinetics

BY T. R. E. KRESSMAN AND J. A. KITCHENER

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A study has been made of the kinetics of exchange between solutions of various simple inorganic and substituted quaternary ammonium salts and the ammonium form of the sulphonated phenol-formaldehyde resin for which equilibrium measurements have been reported elsewhere.

Two mechanisms are observed, where the rate is controlled by diffusion in the particles of the exchanger (*P*-mechanism) and in the bounding Nernst film (*F*-mechanism) respectively. These are distinguished by the form of the kinetics, by interruption tests and by the influence of stirring. The factors that decide which mechanism applies in a given system are discussed.

Measurements of the influence of temperature suggest that so long as the cation is small compared with the pores of the resin (e.g.,  $\text{Na}^+$  and  $\text{NMe}_4^+$  with the present resin) the energy of activation for diffusion is *ca.* 5 kcal./mole as for free diffusion in water. Larger ions, which have difficulty in penetrating, show a higher value, e.g., 8 kcal./mole with  $\text{PhNMe}_2\text{CH}_2\text{Ph}^+$ .

Exchange occurs readily between a granular resin and a finely divided resin in suspension, proving that soluble anions are not needed to release cations from the resin. The high mobility of the cations is consistent with a diffuse double-layer model.

The potentialities of ion exchange separation techniques can be fully exploited only if consideration is given to both equilibrium and kinetic aspects. These are largely independent and they differ with different ion exchangers even though the exchange groups may be the same. There is no simple relationship between affinities and rates of exchange for a series of ions, although with the phenolsulphonate resin used in the present study the rate generally decreases as the affinity increases.

The possibilities of separations based on differences of *rates* of exchange have not yet been fully explored. Fig. 1 shows an example where such an effect might be employed: the concentrations of  $H^+$ ,  $NH_4^+$  and  $NEt_4^+$  in a solution (initially equimolar with respect to  $NH_4^+$  and  $NEt_4^+$ ) are shown during the course of an exchange experiment with a H-resin (containing  $-SO_3^-$  groups) in a stirred system. It is seen that the equilibrium affinity of  $NEt_4^+$  for the resin is greater than that of  $NH_4^+$  but the *rate* of exchange is greater for the  $NH_4^+$  ion. If the experiment were stopped after 2 or 3 min. the solution would be relatively enriched in  $NEt_4^+$ , but at equilibrium the reverse would apply.

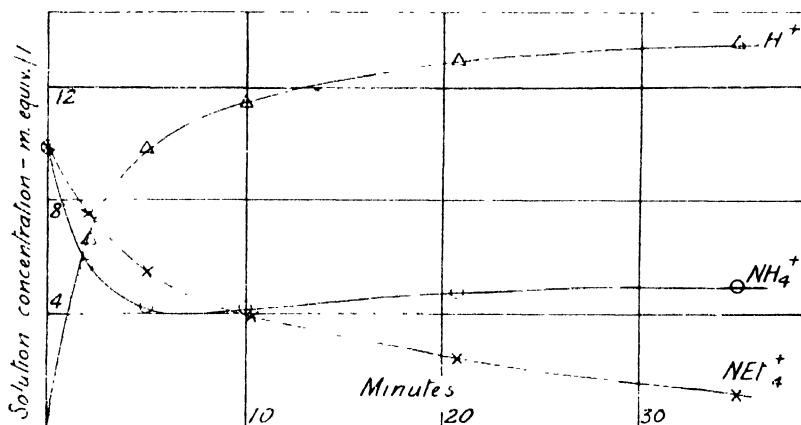


FIG. 1.

Both equilibria and kinetics for a series of ions depend very greatly on the structure of the solid exchanger. The equilibrium relationships for a phenolsulphonate resin similar to Zeo-Karb 215 have already been described elsewhere in Parts I-IV of this series of papers.<sup>1</sup> The present paper describes studies of the kinetics of exchange with the same resin.

### Experiments and Results

**Materials.**—The sample of resin was taken from the same batch of H-resin as that used in earlier studies.<sup>1</sup> The general technique of converting the material to the salt form and of determining the equivalent weights, air drying, etc., is the same as described there. With the exception of the experiments illustrated in Fig. 1, where the H-form of the resin was used, the  $NH_4$ -form was used throughout the work and, after preparing it from the H-resin, it was air-dried and sieved — 10 + 22 mesh.

Two samples were sieved out and all the results except those illustrated in Fig. 8 were obtained with one of these samples. Those in Fig. 8 were obtained with the second sample and, although the resin granules were also — 10 + 22 mesh, a larger fraction of the larger mesh size was present, and the velocity of exchange is thus somewhat lower with this sample than with the first.

<sup>1</sup> Kressman and Kitchener, *J. Chem. Soc.*, 1949, 1190.



In the series of experiments illustrated in Fig. 1 the H-form of the resin was used and, because of its slight instability,<sup>1</sup> it was prepared by thoroughly washing the free acid from a sample taken from the main batch, air-drying rapidly and sieving — 10 + 22 mesh; it was then used immediately. In this way the experiments were completed before the H-resin had begun to hydrolyze.

**Determination of Effective Sphere Radius of the Swollen Resin Granules.**—A large number of particles—of the order of 500—were counted, allowed to swell in water, the water removed and the resin granules mopped between filter papers until the surface moisture had been removed. The swollen granules were then weighed. The density of the swollen granules was determined with the aid of a density bottle in the usual way. The effective sphere radius  $r$  of the swollen granules is calculated from

$$4/3 \cdot \pi r^3 \rho N = W,$$

where  $\rho$  is the density of the swollen resin,  $N$  the number of granules and  $W$  the weight of the  $N$  granules.

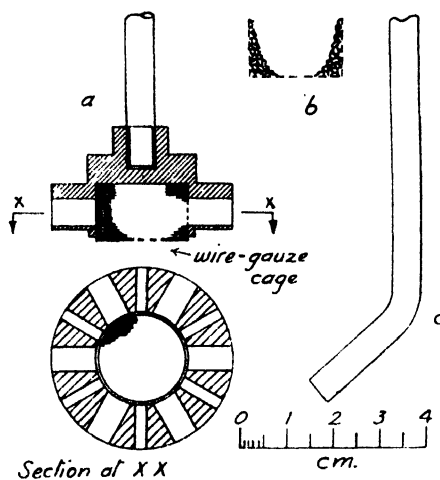


FIG. 2.

The first sample of  $\text{NH}_4$ -resin, which was used for the majority of the work (see under Materials), was found in this way to have an effective sphere radius of 0.446 mm.; and the second sample, used for the experiments illustrated in Fig. 8, an effective sphere radius of 0.517 mm. The density of the swollen granules was 1.33 g./cm.<sup>3</sup>

**Determination of Exchange Velocity.**—The limited bath method was used throughout the work and not the column technique with infinite bath as used by Boyd *et al.*<sup>2</sup> The essential requirements in such heterogeneous studies of standardized vigorous agitation and the necessity of starting and stopping the reaction sharply, were achieved by using a centrifugal type of stirrer as shown in Fig. 2 a. The resin was placed in the wire-gauze cage and as the stirrer rotated in the solution, a very rapid stream of the solution passed over the resin, which was forced into a roughly cylindrical "wall" on the inside of the cage (see Fig. 2 b).

The constancy of speed of rotation of the stirrer was followed stroboscopically. By using several stroboscope discs constant speeds of rotation from 300 rev./min. at intervals to 1200 rev./min. were attained. The reaction was started by lowering the already rotating stirrer, with the resin in the cage, into the solution; and it was stopped after the appropriate interval of time by raising it, still rotat-

<sup>2</sup> Boyd, Adamson and Myers, *J. Amer. Chem. Soc.*, 1947, **69**, 2836.

ing, out of the solution. An aliquot portion of the solution was then analyzed for  $\text{NH}_4^+$  or  $\text{H}^+$  as appropriate. Temperatures between  $14^\circ$  and  $45^\circ \text{C}$  were thermostatically controlled to within  $\pm 0.2^\circ$ ; outside this range the limits were  $\pm 0.5^\circ$ .

The weight of resin taken in every run was such that it contained 2.5 m.equiv. of exchangeable cation, and 2.5 m.equiv. of the other cation were also present in the aqueous phase in the form of 125 ml. 0.02 N solution.

Fig. 3 shows that the mixing is at a maximum when the speed of rotation is between 1000 and 1100 rev./min.; higher speeds caused entrainment of air and, accordingly, an apparent decrease in velocity. The speed of rotation was, therefore, standardized at 1000 rev./min. throughout the work.

**Influence of Agitation.**—Fig. 3 shows how the time for half-change depends upon the speed of rotation of the stirrer for two systems,  $\text{NH}_4\text{R} + \text{NaCl}$  and  $\text{NH}_4\text{R} + \text{NEt}_4\text{Br}$  respectively. The time for half-change in the first system

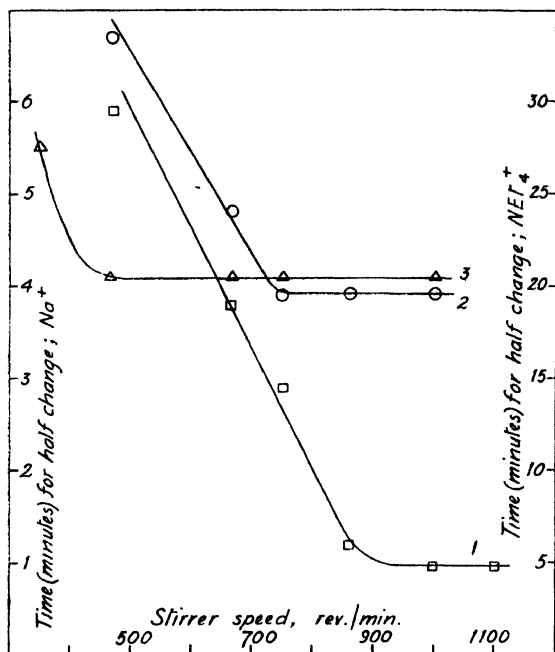


FIG. 3.—Effect of stirring on the time for half-change.

1. Centrifugal stirrer,  $\text{Na}^+$ .
2. Centrifugal stirrer,  $\text{NEt}_4^+$ .
3. Glass stirrer,  $\text{NEt}_4^+$ .

decreases as the speed of rotation is increased, approaching a minimum at 1000 rev./min. when the stationary liquid film surrounding the particles has reached a minimum. (Above about 1100 rev./min. entrainment of air occurs and erratic results are consequently obtained.) No greater velocity was obtained even when the solution was circulated over the resin (in the form of a shallow bed) extremely rapidly by means of a pump.

The time for half-change in the  $\text{NEt}_4^+$  system is also practically at a minimum at 1000 rev./min. but, unlike the  $\text{Na}^+$  system, it remains at a minimum at speeds down to 750 rev./min. and only then, when very little relative motion of solid and liquid is occurring, does it begin to increase.

Fig. 3 also shows a curve obtained in the  $\text{NEt}_4^+$  system with a simple bent glass-rod stirrer (Fig. 2c). This stirrer is seen to be more efficient at slower speeds of rotation but at higher speeds the centrifugal stirrer results in a thinner

liquid film than does the simple stirrer. Evidently, so long as the glass stirrer keeps the particles in suspension, increased agitation has an immeasurable effect upon the thickness of the liquid film.

**Preparation of  $\text{NEt}_4$ -resin in Fine Suspension.**—A series of rate experiments was carried out in which no soluble anions whatever were present in the system, the exchange occurring between the ordinary  $\text{NH}_4$ -resin and a fine aqueous suspension of  $\text{NEt}_4$ -resin. This suspension was prepared by milling well-washed  $\text{NEt}_4$ -resin with water.

A sample of H-resin was washed free of acid and converted to the  $\text{NEt}_4$ -form with a solution of  $\text{NEt}_4\text{Br}$ . The rate of reaction is considerably less than with ammonium chloride and a lower rate of flow was accordingly used, and a greater volume of solution was found necessary to remove the whole of the exchangeable hydrogen ions from the resin. The resin—about 40 g.—was thoroughly washed to remove all traces of electrolytes and then ground in a laboratory porcelain ball mill for about 24 hr. with about 1 l. water. The milky suspension so obtained was allowed to settle for about 1 hr. and the liquid decanted. This consisted of a suspension of the finest particles, the largest of which were of the order of  $1 \mu$  diam. It was adjusted by dilution to contain 0.02 equiv. of exchangeable  $\text{NEt}_4^+$  per l. of suspension, as indicated by a Kjeldahl nitrogen determination.

Care was taken when using this suspension to ensure that homogeneous samples were taken from it. It was vigorously shaken just prior to, and kept gently agitated during, the removal of the sample. The  $\text{NH}_4$ -resin was washed with water before each run was started to remove any traces of electrolytes which might be contaminating it.

The velocity determination with this suspension was carried out exactly as with the aqueous salt solutions. The results obtained are shown in Fig. 5, curve 10, and Fig.

11, curve 6, and graphs of  $Q_t/Q_\infty$  against  $t^\dagger$  for the  $\text{NEt}_4$ -resin suspension and for  $\text{NEt}_4\text{Br}$  indicate that the apparent rate constants are in the ratio of 0.74/1.

**Preparation of the Salts of Chlorazol Sky Blue FFS.**—The sodium salt was prepared from the crude dye by dissolving it in water and salting-out with sodium acetate. This was repeated three times. Adhering sodium acetate was finally removed by washing repeatedly with alcohol. The purified product was dried at  $100^\circ \text{C}$ .

The  $\text{NEt}_4$ -salt was prepared in solution from  $\text{NEt}_4\text{OH}$  and the pure dye acid. The  $\text{NEt}_4\text{OH}$  was prepared in known concentration (about 0.2 N) by adding excess moist silver oxide to a solution of  $\text{NEt}_4\text{Br}$  and filtering. The dye acid was prepared from the pure sodium salt by passing quantitatively a dilute solution (about 0.01 N) containing 4.96 g. of the salt through a 100 ml. column of Zeo-Karb 215 (The Permutit Co., Ltd.) containing exchangeable hydrogen ions. The solution was then evaporated on the water bath until its concentration was of the order of 0.03 N, cooled and "titrated" with the  $\text{NEt}_4\text{OH}$  to pH 7, using a pH meter. The quantity of  $\text{NEt}_4\text{OH}$  required was exactly that calculated from its known concentration. The solution of the  $\text{NEt}_4$ -dye salt was then diluted to 1 l.

In the runs with the dye salts, as in those with the  $\text{NEt}_4$ -resin suspension, the resin was washed with water before each run was started, to remove traces of electrolytes.

**Velocity with Different Cations.**—Since the equilibrium positions are very different with the various cations, and since only the kinetics are at present being considered, the extent of exchange at time  $t$  is expressed as  $Q_t/Q_\infty$ , i.e., the fraction of the amount of exchange occurring at time  $t$  to that occurring at equilibrium. Fig. 5 shows the graphs of  $Q_t/Q_\infty$  against  $t$  for a number of different

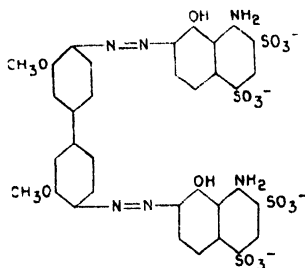


FIG. 4.—Anion of Chlorazol Sky Blue FFS.

cations at 25° C. The same results are plotted in Fig. 6 as  $-\log_{10}(1 - Q_t/Q_\infty)$  against  $t$  and  $Q_t/Q_\infty$  against  $t^{\frac{1}{2}}$  for analysis of mechanism—see below.

**Influence of Temperature.**—The activation energies of the exchange reactions were determined from velocity measurements at several temperatures with the  $\text{NH}_4$ -resin and, respectively,  $\text{Na}^+$ ,  $\text{NMe}_4^+$  and  $\text{PhNMe}_2\text{CH}_2\text{Ph}^+$ .

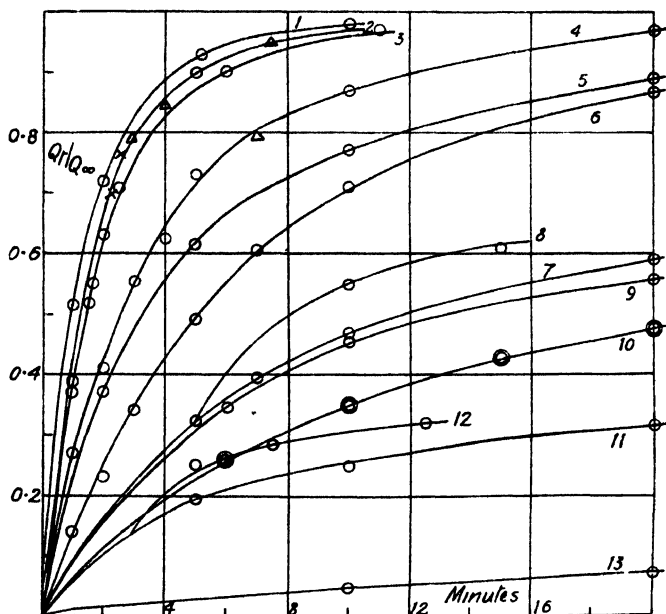


FIG. 5.—Rate of exchange between  $\text{NH}_4$ -resin and various cations at 25° C.

1.  $\text{K}^+$  (chloride).
2.  $\text{Na}^+$  O chloride,  $\times$  interrupted,  $\Delta$  Chlorazol Sky Blue salt.
3.  $\text{Li}^+$  (chloride).
4.  $\text{Mg}^{++}$  and  $\text{Ba}^{++}$ , O chloride,  $\Delta$   $\text{MgSO}_4$ .
5.  $\text{NMe}_4^+$  (bromide).
6.  $\text{Al}^{+++}$  (chloride).
7.  $\text{NEt}_4^+$  (bromide).
8.  $\text{NEt}_4^+$  (bromide) interrupted at 5 min. for 30 min.
9.  $\text{NMe}_3\text{-}n\text{-Amyl}^+$  (bromide).
10.  $\text{PhNMe}_2\text{Et}^+$  (bromide) and  $\text{NEt}_4$ -resin suspension.
11.  $\text{Th}^{++++}$  (nitrate).
12.  $\text{Th}^{++++}$  (nitrate) interrupted at 2.5 min. for 20 min.
13.  $\text{PhNMe}_2\text{CH}_2\text{Ph}^+$  (chloride).

The results are shown in Fig. 7, 8, and 9, where they are plotted in terms of  $-\log_{10}(1 - Q_t/Q_\infty)$  against  $t$ , and  $Q_t/Q_\infty$  against  $t^{\frac{1}{2}}$  as appropriate. The logarithms of the limiting slopes of the lines so obtained are plotted against the reciprocals of the absolute temperature in Fig. 10. The values for the activation energies given in Table I are obtained from the slopes of these straight lines.

TABLE I

Cation	Temperature range	Activation energy kcal./mole
$\text{Na}^+$ (P)	2°–14.8°	5.1
$\text{Na}^+$ (F)	25°–45.7°	5.0
$\text{NMe}_4^+$	1°–61°	5.1
$\text{PhNMe}_2\text{CH}_2\text{Ph}^+$	25°–50°	8.2

### Discussion

The structure of the phenolsulphonate exchange resin is a three-dimensional rigid network resembling a sponge and containing five  $-\text{SO}_3$  groups to every seven phenol residues (see Part I).<sup>1</sup> The cations are highly mobile inside the water-filled interstices, and form a Gouy diffuse double layer round each resin granule.

Consequently, cation exchange does not involve any "chemical" step, since no covalent bond has to be broken, and the rate of exchange depends simply on one or more of a series of consecutive *transport* steps—namely, forced convection of the fluid, diffusion through the unmixed boundary

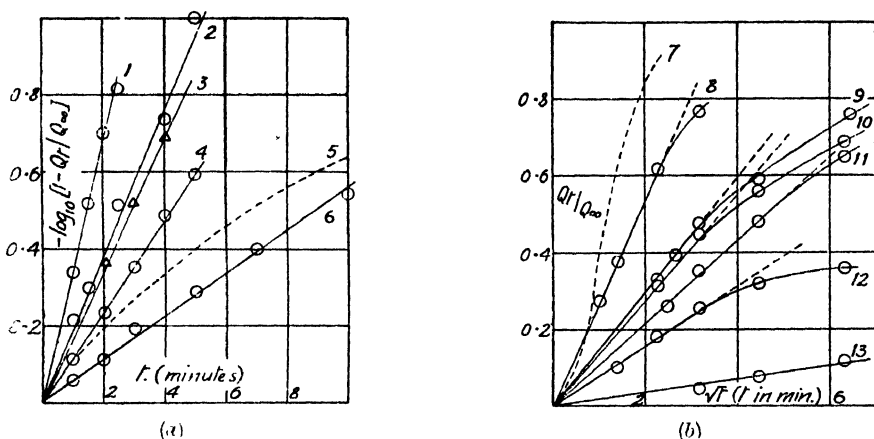


FIG. 6.—Tests of mechanism.

1.  $\text{K}^+$ .
2.  $\text{Li}^+$ .
3.  $\text{Ag}^+$ .
4.  $\text{Mg}^{++}$  and  $\text{Ba}^{++}$ .
5.  $\text{NEt}_4^+$ .
6.  $\text{Al}^{+++}$ .

7.  $\text{Na}^+(25^\circ)$ .
8.  $\text{NMe}_4^+$ .
9.  $\text{NEt}_4^+$ .
10.  $\text{NMe}_2\text{-}n\text{-Amyl}^+$ .
11.  $\text{PhNMe}_2\text{Et}^+$  and  $\text{NEt}_4\text{-resin suspension}$ .
12.  $\text{Th}^{+++}$ .
13.  $\text{PhNMe}_2\text{CH}_2\text{Ph}^+$ .

layer and diffusion inside the particles. As Boyd *et al.*<sup>2</sup> have shown, three possible types of kinetics may therefore be encountered in a practical, dynamic exchange process:

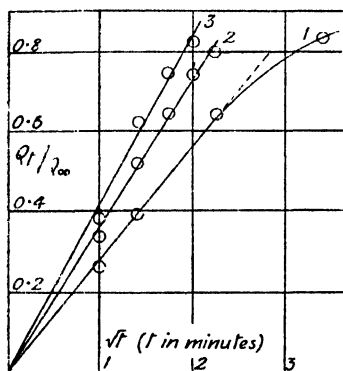
- (i) Diffusion in the boundary liquid film is rate-determining (*F*-mechanism).
- (ii) Diffusion in the solid particle is rate-determining (*P*-mechanism).
- (iii) In an intermediate region the rate is influenced by diffusional resistances in *both* phases (*I*-mechanism).

The factors that may decide which mechanism applies in a given system are (1) particle size, (2) degree of agitation of the solution, (3) diffusion coefficients of the ions in the solution and inside the resin particles, (4) temperature, (5) the equilibrium distribution coefficient and (6) solution concentration. Variation of any one of these factors may produce a change of mechanism. For example, Boyd *et al.*<sup>2</sup> have shown that the  $\text{Na}^+ - \text{K}^+$  exchange (under their conditions of experiment) is *F* at 0.001 M ionic strength, *I* at 0.01 M and *P* at 0.1 M.

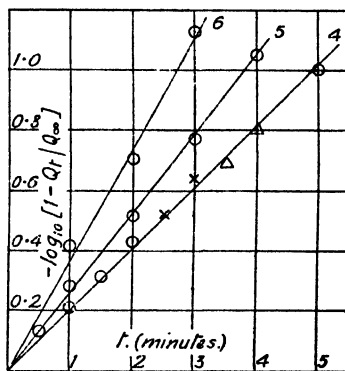
### Criteria for Distinguishing Mechanisms.

(a) **Influence of Stirring.**—The rate increases with the degree of mixing of the liquid as long as the *F*- or *I*-mechanisms are rate-controlling, whereas with *P*-mechanism it should be independent. With increasing speed of rotation of a given stirrer, a maximum exchange rate may be reached, beyond which the rate may remain constant or even decrease (Fig. 3). Only exceptionally does such a maximum indicate the point where *I*-mechanism gives place to *P*. More usually, the rate reaches a limit for hydrodynamical reasons, the stirrer having reached its limiting efficiency for mixing, and it may still be possible to achieve faster rates of exchange by using a different type of stirrer (Fig. 3).

However, in border-line cases a change of mechanism may sometimes be detected by the influence of stirring—notably, when a maximum exchange rate is reached in the stirring range slower than the known hydrodynamical limit. The exchange with  $\text{NEt}_4^+$  in Fig. 3 provides an instance of this.



(a)



(b)

FIG. 7.—Influence of temperature,  $\text{Na}^+$ .

1.  $2.0^\circ \text{C}$
2.  $8.4^\circ$
3.  $14.8^\circ$

4.  $25.0^\circ \text{C}$
5.  $34.8^\circ$
6.  $45.7^\circ$

- Chloride  
 × interrupted  
 Δ Chlorazol Sky Blue salt

(b) **Form of the Kinetics.**—(i) **FILM DIFFUSION (*F*-MECHANISM).**—The well-known Nernst static diffusion film theory is clearly only a crude approximation for the complex situation which exists near an irregular solid surface in a stirred liquid; in particular, the “thickness” of such a layer is a mathematical fiction which has proved of little use since this quantity must usually be deduced from the kinetics (not vice versa), and its dependence on stirring, temperature, viscosity, etc., is problematical. Only in a few idealized cases has it been possible to calculate the diffusional transport up to a body in a stirred liquid.<sup>3</sup> However, the Nernst layer approximation is useful for treating the kinetics of exchange under fixed conditions of stirring and temperature.<sup>2</sup>

In the present system, let  $Q_0$  be the number of milli-equivalents of the pure  $\text{A}^+$  form of the resin, which at time  $t = 0$  is brought into contact with  $V$  ml. solution containing  $Q_0$  milli-equivalents of a salt of the cation  $\text{B}^+$ . Suppose the resin consists of  $n$  particles of mean equivalent sphere radius  $r$ ,

<sup>3</sup> Levich, *Acta Physicochim.*, 1942, **17**, 257; 1944, **19**, 117, 133; *Faraday Soc. Discussions*, 1947, **1**, 37.

and that the effective Nernst diffusion layer thickness is  $\delta$ . Let the amount of exchange which has occurred after time  $t$  be  $Q_t$  (milli-equivalents). The diffusion process taking place in the Nernst layer is essentially a cation exchange at constant ionic strength. The rate is therefore proportional to the gradient of concentration of  $A^+$  (or  $B^+$ ); let  $D_L$  be the Fick's law diffusion coefficient for the inter-diffusion of  $A^+$  and  $B^+$  under these conditions.

After time  $t$  the concentration of  $A^+$  on the outer side of the Nernst layer is  $Q_t/V$ . The concentration of  $A^+$  at the surface of the particle is more difficult to estimate. Boyd *et al.*<sup>2</sup> took this as the concentration which would be found in a solution in equilibrium with the prevailing resin composition. Under their conditions this was simply proportional to the  $[A^+]$  in the resin, but under the present conditions it would be necessary to introduce the mass action equilibrium constant

$$K = \left( \frac{[A^+]}{[B^+]} \right)_{\text{soln.}} \times \left( \frac{[B^+]}{[A^+]} \right)_{\text{resin}}$$

(see Part I),<sup>1</sup> and the resulting kinetics would be mathematically very complicated.

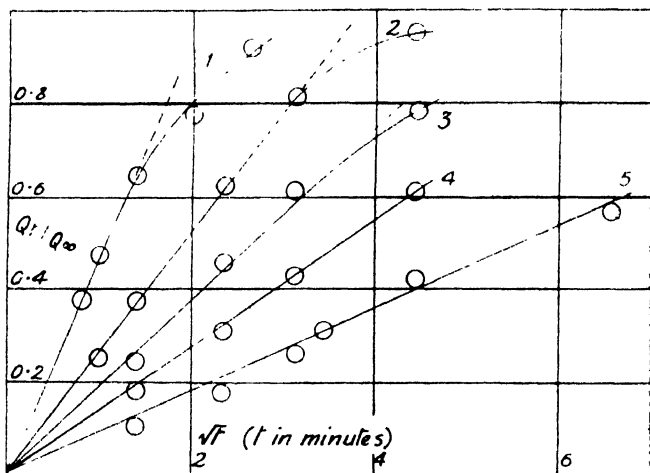


FIG. 8.—Influence of temperature,  $NMe_4^+$ .

- |            |            |
|------------|------------|
| 1. 60.6° C | 4. 12.6° C |
| 2. 41.3°   | 5. 1.2°    |
| 3. 25.0°   |            |

However, it will be shown that the cations in the exchanger are highly mobile; there seems therefore to be no reason to distinguish between "free" and "bound" cations in the resin phase—at least for simple inorganic cations. Further, the number of cations present at the outer surface of the particles as gegenions to the  $-SO_3^-$  groups far exceeds those present as soluble electrolyte from the solution phase since soluble anions are largely repelled by the opposing zeta potential.<sup>4</sup> Consequently, the number of  $A^+$  ions which are free to participate in diffusion away from the surface of the sphere at any instant is simply proportional to the number present in the resin: let it be put equal to  $k'(Q_0 - Q_t)$ .

<sup>4</sup> See, for example, Verweg and Overbeek, *Theory of the Stability of Lyophobic Colloids*, (Elsevier, 1948), p. 31.

Application of Fick's law to the Nernst layer (the diffusion gradient being assumed linear) gives

$$\frac{dQ_t}{dt} = \frac{D_L}{\delta} \left[ k'(Q_\infty - Q_t) - \frac{Q_t}{V} \right].$$

Integration leads finally to the equation

$$\frac{Q_\infty}{Q_0} \ln \left( 1 - \frac{Q_t}{Q_\infty} \right) = kt,$$

where  $Q_\infty$  is the amount of  $A^+$  which has passed into the solution when equilibrium has been reached, and  $k$  is a constant, equal to  $Dk'/\delta$ .

This equation is of the same form as that obtained by Boyd *et al.*<sup>2</sup> (for an infinite bath), which was found to fit the kinetics of their  $Na^+-K^+$  exchange in dilute solutions at least up to  $Q_t/Q_\infty = 0.4$ . In the present work the equation fits the kinetics well in appropriate cases (see Fig. 6 *a* and 7 *b*). For example, the  $NH_4^+-Na^+$  exchange at 25° C fits accurately from  $Q_t/Q_\infty = 0$  up to at least  $Q_t/Q_\infty = 0.9$ . Other cases which conform to these kinetics include  $Ag^+$ ,  $K^+$ ,  $Li^+$ ,  $Mg^{++}$ ,  $Ba^{++}$ ,  $Al^{+++}$ .

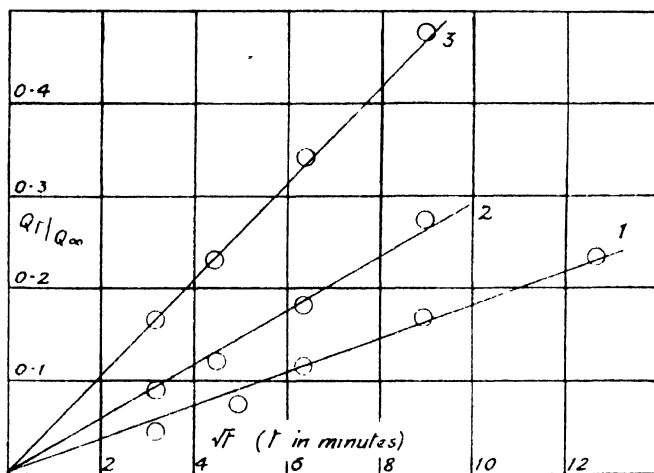


FIG. 9.—Influence of temperature,  $PhNMe_2CH_2Ph^+$ .  
1. 25.0° C                      2. 36.5° C                      3. 50.0° C

(ii) PARTICLE DIFFUSION (*P*-MECHANISM).—Assuming the solution effectively uniform up to the particle surface and the rate controlled by radial diffusion inwards with a constant diffusion coefficient, the kinetics of the *P*-mechanism should be formally similar to those for conduction of heat into a sphere from a well-stirred bath. Boyd *et al.*<sup>2</sup> have successfully applied the well-known theory for conduction of heat into a sphere from an infinite bath. The corresponding theory for a limited bath is given by Carslaw and Jaeger.<sup>5</sup> The form of their solution, however, is less convenient than that of an alternative solution recently given by Paterson,<sup>6</sup> viz.,

$$\frac{Q_t}{Q_\infty} = \frac{w + 1}{w} \left\{ 1 - \frac{1}{\alpha - \beta} \left[ \alpha e^{\alpha^2 \tau} (1 + \operatorname{erf} \alpha \sqrt{\tau}) - \beta e^{\beta^2 \tau} (1 + \operatorname{erf} \beta \sqrt{\tau}) \right] \right\},$$

<sup>5</sup> Carslaw and Jaeger, *Conduction of Heat in Solids* (Oxford Univ. Press, 1947), pp. 83, 201.

<sup>6</sup> Paterson, *Proc. Phys. Soc.*, 1947, **59**, 50.



where  $\tau = \frac{\kappa t}{r^2}$ ,  $\kappa$  being the thermal diffusivity; and  $\alpha$  and  $\beta$  are the roots of the equation

$$x^2 + 3wx - 3w = 0$$

and  $w$  is (heat capacity of the sphere)/(heat capacity of the bath). This solution is valid up to  $\tau = 0.1$  which, in practice, covers most of the process (e.g., up to  $Q_t/Q_\infty = 0.84$ , when  $w = 1$ ).

In attempting to apply Paterson's solution to the ion-exchange process with, e.g.,  $\text{NEt}_4^+$  and  $\text{NH}_4$ -resin,  $\kappa$  is identified with the cation-exchange diffusion coefficient inside the resin ( $D_P$ ), and  $Q_t/Q_\infty$  with the ratio ( $\text{NH}_4^+$  out in time  $t$ )/( $\text{NH}_4^+$  out at equilibrium), and  $w$  with the ratio ( $\text{NEt}_4^+$  in

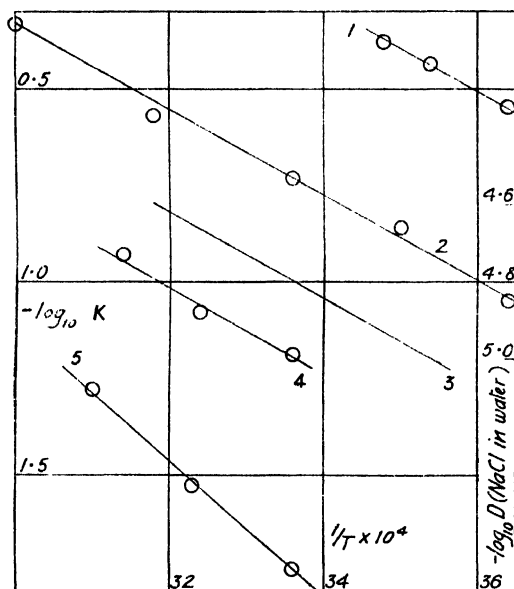


FIG. 10.—Activation energy data.

1.  $\text{Na}^+$  (P).
2.  $\text{NMe}_4^+$ .
3. Diffusion coefficient data for NaCl in water.<sup>7</sup>
4.  $\text{Na}^+$  (F) (−0.5 added to vertical scale).
5.  $\text{PhNMe}_2\text{CH}_2\text{Ph}^+$ .

resin at equil.)/( $\text{NEt}_4^+$  in solution at equil.). From the value of  $w$ ,  $\alpha$  and  $\beta$  can be calculated and a graph of  $Q_t/Q_\infty$  as a function of  $\tau$  can be computed. Since

$$\tau = (D_P/r^2)t,$$

the best value of  $(D_P/r^2)$  can be found to fit the theoretical curve to the experimental points (provided, of course, the theory gives the correct form of the kinetics); hence,  $r$  being determined independently, a value of  $D_P$  can be obtained.

The attempt to fit the equation in this way to the data in Fig. 11 did not lead to satisfactory agreement: when experiment and theory were fitted at  $Q_t/Q_\infty = 0.5$ , the deviations were always in the sense that the equation predicted a more rapid reaction initially than in fact occurred. Barrer<sup>8</sup>

<sup>7</sup> *Int. Crit. Tables*, Vol. 5.

<sup>8</sup> Barrer, *Trans. Faraday Soc.*, 1949, **45**, 358.

has recently discussed possible causes for deviations in the somewhat analogous system of gas sorption by zeolites. Lack of conformity in the present system could be due to (1) slow swelling of the initially dry resin, and (2) change of diffusion coefficient with resin composition. (Curiously enough, good agreement was obtained when  $w$  was taken as  $(\text{NH}_4^+ \text{ in resin at equil.})/(\text{NH}_4^+ \text{ in solution at equil.})$ ; the lines in Fig. 11 were obtained in this way. The significance, if any, of this is not clear.)

Barrer<sup>8</sup> has shown that Paterson's solution, like the well-known one for diffusion into a sphere from an infinite bath, approximates to a  $t^{1/2}$ -relation for small values of  $Q_t/Q_\infty$ , the limiting expression being

$$\frac{Q_t}{Q_\infty} = \frac{6}{r} \cdot \frac{Q_0}{Q_\infty - Q_0} \cdot \sqrt{\frac{Dt}{\pi}}$$

However, examination of such graphs shows that whereas the line for an infinite bath is within 2 % of linear up to about  $Q_t/Q_\infty = 0.5$ , the linear range becomes progressively shorter as the bath becomes more limited. Thus, at  $w = 1$  the  $t^{1/2}$  graph is linear only within 4 % up to  $Q_t/Q_\infty = 0.25$ . Consequently, in testing for  $P$ -mechanism by this method of plotting the data, a straight line is to be expected only at small values of  $Q_t/Q_\infty$ . Fig. 6 *b* and 7 *a* show examples of exchange reactions which conform approximately to these expectations. Values for the nominal diffusion coefficients obtained from these graphs are given in Table II.

The  $t^{1/2}$  graph is used as the first test for  $P$ -mechanism. It is fortunate that the  $F$ - and  $P$ -kinetics are sufficiently different in form to be distinguished readily by conformity to either  $\log(1 - Q_t/Q_\infty)$  against  $t$  or  $Q_t/Q_\infty$  against  $t^{1/2}$  graphs respectively. Fig. 6 (curves 5 and 7) shows how data which fit one are clearly excluded from fitting the other.

(iii) **INTERMEDIATE ( $I$ -MECHANISM).**—The full kinetics of the  $I$ -process, transitional between  $F$ - and  $P$ -, have not yet been worked out for a limited bath. The analogous problem of heat conduction into a composite sphere has been solved for the case of a constant surface temperature.<sup>9</sup> However, the algebra is already very complicated, and with a finite bath would be much more so. Crank and Godson<sup>10</sup> have obtained approximate numerical solutions for certain cases of composite infinite cylinders in a limited bath using the method of finite differences. The same method could be used for the present problem, but it would be necessary to have more explicit knowledge of the constant  $k'$  in the  $F$ -kinetics theory than is at present available.

The only example of what appears to be  $I$ -mechanism encountered in the present work is the  $\text{Na}^+ - \text{NH}_4^+$  exchange within the range 15°–25° C, the kinetics at 25°, 35° and 46° fitting  $I'$ -mechanism and those at 15°, 8° and 2° fitting  $P$ -mechanism.

(c) **Interruption tests**<sup>11</sup> provide a simple criterion for the existence of a large concentration gradient inside the particles, thus differentiating  $P$ - or  $I$ -mechanism from  $F$ . In the present work such tests have, in every case where applied, confirmed the mechanisms already suggested from the

TABLE II  
DIFFUSION COEFFICIENTS AT 25° C

Cation	$D_p(\text{cm.}^2\text{sec.}^{-1} \times 10^8)$
$\text{NMe}_4^+$	2.4
$\text{NEt}_4^+$	0.5
$\text{NMe}_3 \cdot n\text{-Amyl}^+$	0.3
$\text{PhNMe}_2\text{Et}^+$	0.1
$\text{PhNMe}_2\text{CH}_2\text{Ph}^+$	0.006

<sup>9</sup> Carslaw and Jaeger, ref. 5, p. 288.

<sup>10</sup> Crank and Godson, *Phil. Mag.*, 1947, **38**, 794.

<sup>11</sup> Kunin and Myers, *J. Physic. Chem.*, 1947, **51**, 1111.

form of the kinetics. Thus, the  $\text{Na}^+-\text{NH}_4^+$  exchange at  $25^\circ$  gives  $F$ -kinetics and shows no discontinuity on interruption (Fig. 7, curve 4) whereas  $\text{Na}^+-\text{NH}_4^+$  at  $2^\circ$ , and  $\text{NMe}_4^+$  and  $\text{NEt}_4^+-\text{NH}_4^+$  at  $25^\circ$  give  $P$ -kinetics and show a large discontinuity in the expected direction. Likewise  $\text{Th}^{++++}-\text{NH}_4^+$  gives  $P$ -kinetics and shows a similar discontinuity on interruption (see Fig. 5, curves 8 and 12).

(d) **Temperature coefficient** might be expected to afford a criterion of mechanism, since the temperature coefficient for diffusion through the solid might reasonably be supposed to be distinctly higher than for free diffusion in solution. This is certainly the case with large molecules such as dyes penetrating into fibres, where the activation energy is about

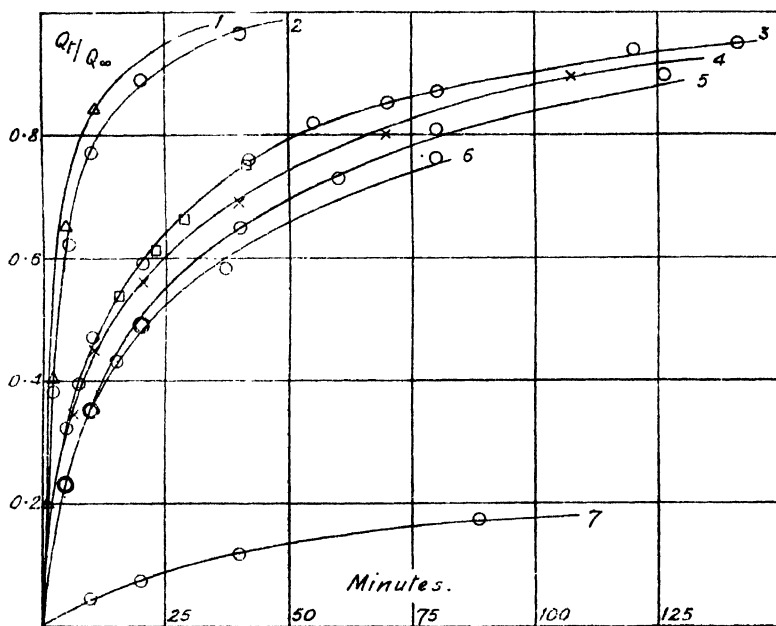


FIG. 11. Systems showing particle diffusion.

- |   |   |
|---|---|
| 1. $\text{Na}^+$ $2^\circ \text{C}$                         | 5. $\text{PhNMe}_2\text{Et}^+$ $25^\circ \text{C}$            |
| 2. $\text{NMe}_4^+$ $25^\circ \text{C}$                     | 6. $\text{NEt}_4^+$ -resin suspension $25^\circ \text{C}$     |
| 3. $\text{NEt}_4^+$ $25^\circ \text{C}$ . $\circ$ bromide   | 7. $\text{PhNMe}_2\text{CH}_2\text{Ph}^+$ $25^\circ \text{C}$ |
| 4. $\text{NMe}_4$ - <i>n</i> -amyl $^+$ $25^\circ \text{C}$ | $\square$ Chlorazol Sky Blue salt                             |

10–30 kcal./mole,<sup>12</sup> whereas that for diffusion of most salts in water is about 5 kcal./mole.<sup>7</sup> Similarly, Boyd *et al.*<sup>2</sup> found 4 kcal./mole for  $\text{Na}^+$  ( $F$ -mechanism) and 8 kcal./mole for  $P$ -mechanism with Amberlite IR-1.

Table I shows values of the energy of activation for several exchange reactions studied in the present work (see also Fig. 10). The exchange between  $\text{NH}_4^+$ -resin and  $\text{Na}^+$  at  $25^\circ$ – $46^\circ$  ( $F$ -) shows the expected value of about 5, but it is remarkable that both  $\text{Na}^+$  at  $2^\circ$ – $15^\circ$  ( $P$ -) and  $\text{NMe}_4^+$  ( $P$ -) have the same low value. A high value of 8.2 kcal./mole is found for  $\text{PhNMe}_2\text{CH}_2\text{Ph}^+$ . This is the largest ion yet studied which is capable of

<sup>12</sup> See, e.g., Speakman and Smith, *J. Soc. Dyers Col.*, 1930, **52**, 121. Garvie, Griffiths and Neale, *Trans. Faraday Soc.*, 1934, **30**, 271.

penetrating and reaching all the exchanging sites in this resin and it seems likely that the higher activation energy is a consequence of the steric difficulty which it experiences in moving through the molecular pores of the resin. On the other hand, the smaller ions showing *P*-mechanism evidently do not experience any such "wall-effect" and move simply through the water which fills the pores.

It is not invariably true that diffusion in solids requires large energies of activation, and results in agreement with the present picture have been obtained by other workers. The high value with fibres may reflect distortion of a flexible macromolecular structure by the dye molecule. With rigid porous solids, the activation energy for the diffusion of molecules *smaller than the pores* may be almost normal for an aqueous medium. For example, Tiselius<sup>13</sup> found 5.4 kcal./mole for the diffusion of water normal to the (201) face in the zeolite, heulandite. On the other hand, diffusion normal to the (001) face required 9.1 kcal/mole; presumably the interstitial holes in this direction are much smaller, and of about the same size as the water molecule. Diffusion of salts in 2% agar gels shows the same temperature coefficient as for free diffusion in aqueous solution.<sup>7</sup>

**Role of the Anion.**—To preserve electro-neutrality, the ions leaving the resin must be replaced by others of the same total charge. If the ions were bound to specific sites the situation might arise where the rate of dissociation from sites was the factor limiting the rate of exchange, and it might then be necessary to provide extra (soluble) anions by diffusion of salt from the ambient solution into the interstices of the resin before rapid cation exchange could take place.

This possibility was studied by the experiments (recorded above) in which the anion was provided by the dye Chlorazol Sky Blue FFS, which is found to diffuse extremely slowly and to an extremely small extent into the resin. With both  $\text{Na}^+$  and  $\text{NEt}_4^+$  the rate of exchange was the same as with the simple halide salts (see Fig. 5, curve 2; Fig. 7, curve 4; Fig. 11, curve 3), indicating that it is not necessary to have soluble anions within the pores for exchange to occur rapidly.

The high mobility of the cations alone is proved conclusively by the experiment with finely divided  $\text{NEt}_4$ -resin *suspension* in place of  $\text{NEt}_4\text{Br}$  solution. This suspension exchanges cations with normal  $\text{NH}_4$ -resin grains almost as rapidly as does the homogeneous salt solution, the apparent rate constants being in the ratio of 0.74/1 (cf. Fig. 5, curves 7 and 10), proving that soluble anions play no significant part at all in the kinetics of cation exchange, at least with the resinous exchanger studied here.

In this experiment the process starts with the interpenetration of the Gouy diffuse double layers of the large grains with those of the small suspended particles. At the very low ionic strengths prevailing, the double layers spread so far from the microscopic particles that the cation distribution in the liquid is similar to that in a true salt solution. Consequently, the outside part of the double layer from the large granules can mix rapidly with the cations of the suspension, leaving diffusion of cations within the pores of the resin as the rate-controlling process, as it is with the ordinary salt solution. A similar process no doubt occurs in normal exchanges with soluble salts—the soluble cations exchange first with the resin cations in the outer diffuse double layer, thus setting up a concentration gradient within the pores which is eliminated by ordinary diffusion, this latter process requiring no soluble anions.

<sup>13</sup> Tiselius, *Z. physik. Chem. A*, 1934, **169**, 425; 1935, **174**, 401.

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## ION EXCHANGE STUDIES

### II. The Determination of Thermodynamic Equilibrium Constants

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A critical examination of the postulates used by different authors to evaluate the activities of ions in an exchanger is given and an experimental test is made using the barium-hydrogen and lanthanum-ammonium exchange systems. It is shown that the assumption that the activities in the exchanger are proportional to the molar concentrations (in arbitrary units) gives an approximately constant value for the mass product, but even on this postulate the mass product varies by about 50 %, rising to a maximum at an equivalent fraction of about  $\frac{1}{4}$  for the multivalent ion in the exchanger. It is suggested that the activities in the exchanger have been wrongly evaluated because of the influence of some secondary process, such as adsorption, solution of ions in the exchanger or swelling of the resin.

In this paper the following symbols have been used :

$a$ , thermodynamic activity.

$K_a$ , thermodynamic equilibrium constant.

$c$ , molar concentration.

$x$ , molar fraction.

$C$ , equivalent concentration.

$X$ , equivalent fraction, defined as the ratio of the number of equivalents of a given cation to the total number of equivalents of all cations in the same phase.

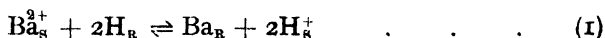
$n$ , number of moles.

Suffixes have been used to indicate the ion and the particular phase to which reference is made, thus

$x_{Ba_R}$  is the molar fraction of barium ions in the resinous exchanger.

$a_{H_S}$  is the thermodynamic activity of hydrogen ions in solution.

The thermodynamic equilibrium constant of an ion-exchange reaction of the type



is written

$$K_a = \frac{a_{Ba_R}}{a_{H_R}^2} \cdot \frac{a_{H_S}^2}{a_{Ba_S}} \quad . \quad . \quad . \quad (2)$$

Whilst the activities  $a_{Ba_S}$ ,  $a_{H_S}$  in solution may be readily evaluated in favourable cases from known activity data, some assumption must be made for the activities of the two ions in the solid phase. Hitherto, at least three different assumptions have been made for  $a_{Ba_R}$  and  $a_{H_R}$  as follows :

(i) Boyd, Schubert and Adamson<sup>1</sup> have assumed that the ions in the solid phase exist in ideal solution, and have put the activities in the solid phase equal to the molar fractions of the two species. If the  $x$  terms are molar fractions and the  $n$  terms are the number of moles of  $\text{Ba}_R$  and  $\text{H}_R$ , then

$$K_a = \frac{x_{\text{Ba}_R}}{x_{\text{H}_R}^2} \cdot \frac{a_{\text{H}_S}^2}{a_{\text{Ba}_S}} = \frac{(n_{\text{Ba}_R} + n_{\text{H}_R}) n_{\text{Ba}_R}}{n_{\text{H}_R}^2} \cdot \frac{a_{\text{H}_S}^2}{a_{\text{Ba}_S}} \quad (3)$$

By plotting  $\log (x_{\text{Ba}_R}/x_{\text{H}_R}^2)$  against  $\log (a_{\text{Ba}_S}/a_{\text{H}_S}^2)$  for the sodium-barium system a straight line was obtained by Boyd, Schubert and Adamson, which seemed to indicate that the concept of ideal solution in the solid phase was a reliable hypothesis.

(ii) Bauman and Eichhorn<sup>2</sup> on the other hand have written the equilibrium constant of a reaction of the above type in a form corresponding to

$$K_a = \frac{c_{\text{Ba}_R}}{c_{\text{H}_R}^2} \cdot \frac{a_{\text{H}_S}^2}{a_{\text{Ba}_S}} \quad (4)$$

where the  $c$  terms are molar concentrations, and have derived an equation of the form

$$\left( \frac{x_{\text{Ba}_R}}{(1 - 2x_{\text{Ba}_R})^2} \right)^2 = K_a \cdot \frac{c_R}{c_S} \cdot \left( \frac{x_{\text{H}_S}}{(1 - 2x_{\text{Ba}_S})^2} \right)^2 \quad (5)$$

where  $c_R$  is the total molar exchange capacity of the exchanger and  $c_S$  the total molar concentration of the solution. It is difficult to see how this equation can be derived from eqn. (4) unless some assumption has been made which has not been specifically mentioned in their paper. Nevertheless, these authors make a test of their equation which appears to support their postulates.

(iii) Kressman and Kitchener<sup>3</sup> have assumed that the activities of the ions in the exchanger are proportional to the equivalent fractions defined as

$$a_{\text{Ba}_R} = \frac{C_{\text{Ba}_R}}{C_R} = X_{\text{Ba}_R}, \text{ and } a_{\text{H}_R} = \frac{C_{\text{H}_R}}{C_R} = X_{\text{H}_R} = 1 - X_{\text{Ba}_R},$$

where the  $C$  terms are the number of equivalents of the ions in the exchanger,  $C_R$  is the total capacity, and the  $X$  terms are the equivalent fractions in the exchanger. Hence

$$K_a = \frac{X_{\text{Ba}_R}}{(1 - X_{\text{Ba}_R})^2} \cdot \frac{a_{\text{H}_S}^2}{a_{\text{Ba}_S}} \quad (6)$$

In this treatment the activity coefficients of the ions in solution have also been neglected, and the equation written

$$K_a = \frac{X_{\text{Ba}_R}}{(1 - X_{\text{Ba}_R})^2} \cdot \frac{C_{\text{H}_S}^2}{C_{\text{Ba}_S}} \quad (7)$$

The mass-law product estimated from an equation of this form was found to be constant over a range of  $0.76 < X_{\text{Ba}_R} < 0.88$  for the barium-ammonium system (and over similar ranges for the other systems), and it was concluded that the changes occurring in  $\gamma_{\text{Ba}_S}/\gamma_{\text{H}_S}^2$  must be accompanied by proportionate changes in  $\gamma_{\text{Ba}_R}/\gamma_{\text{H}_R}^2$ .

It appears surprising that three different assumptions for the activities of the ions in the exchanger can lead to equations which are apparently

<sup>1</sup> Boyd, Schubert and Adamson, *J. Amer. Chem. Soc.*, 1947, **69**, 2818.

<sup>2</sup> Bauman and Eichhorn, *ibid.*, 2830.

<sup>3</sup> Kressman and Kitchener, *J. Chem. Soc.*, 1949, 1201.

equally successful in describing the results. All three approaches when applied to exchanging ions of the same valency lead to the same equations, which would therefore be expected to give a constant mass-law product for homovalent exchange. We have previously shown<sup>4</sup> that the mass-law product for the sodium-hydrogen system is not constant but rises to a maximum for a value of 0.12 for the molar fraction of sodium ions in the exchanger. This suggests that the simple assumptions made for the activities of ions of the same valency in the exchanger is not valid, and, *a priori*, one would expect these assumptions to be in greater error for heterovalent exchange, for which the system is likely to be less ideal.

The situation is further confused by the apparent contradictory nature of eqn. (3) and (4). If the capacity of the exchanger is constant, the postulates of Kressman and Kitchener and of Bauman and Eichhorn are such that a constant mass-law product can be obtained from the same set of results by use of both eqn. (4) and (7) (provided the ratio of the activity coefficients of the ions in solution does not vary greatly), for

$$\frac{X_{\text{BaR}}}{(1 - X_{\text{BaR}})^2} = \frac{C_{\text{BaR}} \cdot C_{\text{R}}}{C_{\text{HR}}^2} = \frac{2c_{\text{BaR}} \cdot C_{\text{R}}}{c_{\text{HR}}^2} \quad (8)$$

The postulates of Boyd, Schubert and Adamson and those of the other two sets of authors are, however, mutually exclusive, for if eqn. (4) is written in terms of the number of moles of the two ions in a given weight of exchanger we arrive at

$$K_a = \frac{n_{\text{BaR}}}{n_{\text{HR}}^2} \cdot \frac{a_{\text{HS}}^2}{a_{\text{BaS}}} \quad (9)$$

This is different from eqn. (3) which has a term  $(n_{\text{BaR}} + n_{\text{HR}})$  equal to the total number of moles of the two ions in the exchanger, which must vary according to the position of equilibrium. Thus it is impossible to obtain a constant mass-law product using eqn. (3) from results which give a constant with eqn. (4) and (6).

In view of this confusion, a thorough test of the mass-action concept has been made by estimating the mass-law product from the same results using eqn. (3), and also by assuming the activities in the exchanger to be equal to the molar concentrations (i.e., neglecting the activity coefficients of the ions in the exchanger). In order to simplify the presentation of the results, eqn. (4) obtained on the latter assumption has been used in the form given by substitution of the equivalent fractions for the concentration terms. Thus

$$\begin{aligned} K_a &= \frac{C_{\text{BaR}}}{C_{\text{HR}}^2} \cdot \frac{a_{\text{HS}}^2}{a_{\text{BaS}}} \\ &= \frac{C_{\text{BaR}}}{2C_{\text{HR}}^2} \cdot \frac{c_{\text{HS}}^2}{C_{\text{BaS}}} \cdot \frac{\gamma_{\text{HS}}^2}{\gamma_{\text{BaS}}} \\ &= \frac{C_{\text{BaR}}}{2C_{\text{HR}}^2} \cdot \frac{2C_{\text{HS}}^2}{C_{\text{BaS}}} \cdot \frac{\gamma_{\text{HS}}^2}{\gamma_{\text{BaS}}} \\ &= \frac{X_{\text{BaR}}}{X_{\text{HR}}^2} \cdot \frac{X_{\text{HS}}^2}{X_{\text{BaS}}} \cdot \frac{\gamma_{\text{HS}}^2}{\gamma_{\text{BaS}}} \cdot \frac{C_{\text{S}}}{C_{\text{R}}} \end{aligned}$$

Hence

$$K_a = \frac{X_{\text{BaR}}}{(1 - X_{\text{BaR}})^2} \cdot \frac{(1 - X_{\text{BaR}})^2}{X_{\text{BaS}}} \cdot \frac{\gamma_{\text{HS}}^2}{\gamma_{\text{BaS}}} \cdot \frac{C_{\text{S}}}{C_{\text{R}}} \quad (10)$$

<sup>4</sup> Duncan and Lister, *ibid.* (in press).

where  $C_s$  is the total equivalent concentration in solution, and the  $X$  terms are equivalent fractions of the respective ions in the exchanger and in solution. The ratio of the  $K_a$  values given by eqn. (10) and (6) is equal to the capacity of the exchanger.

For the barium-hydrogen system, plots of  $K_a$  against  $x_{Ba_R}$  and against  $X_{Ba_R}$  were made to test eqn. (3) and (10) respectively. In both cases the mass-law product was found to vary, but the curve obtained using eqn. (10) was more nearly constant and had a form similar to that obtained for the sodium-hydrogen system. The same was true of the exchange of lanthanum and ammonium ions, the value of  $K_a$  being evaluated according to the equation,

$$K_a = \frac{X_{La_R}}{(1 - X_{La_R})^3} \cdot \frac{(1 - X_{La_R})^3}{X_{La_R}} \cdot \frac{\gamma_{H_R}^3}{\gamma_{La_R}} \cdot \frac{C_s^2}{C_R^2} \quad (11)$$

### Experimental

The position of equilibrium between exchanger and solution has been determined (i) by batch equilibration methods and (ii) by determining the breakthrough volume necessary for a column of exchanger in the hydrogen form to be saturated by a given mixture of metal ions and hydrogen ions. The apparatus was in principle the same as that used for investigating the sodium-hydrogen system.<sup>4</sup>

Radiochemical tracer methods were used to study both the barium and the lanthanum systems, the two tracers being  $^{130}\text{Ba}$  (half-life 86 min.,  $\beta$ -energy 2.3 MeV,  $\gamma$ -energy 0.6 MeV) and  $^{140}\text{La}$  (half-life 40 hr.,  $\beta$ -energy 1.45 MeV,  $\gamma$ -energies 0.87, 0.49 and 0.33 MeV) respectively. In the lanthanum-ammonium batch equilibration method, volumes of radioactive lanthanum nitrate solution from 100 ml. to 5 l. were made up with ammonium nitrate to a total concentration of 0.1 N, the lanthanum concentration being varied from  $10^{-2}$  to  $10^{-6}$  N. To these solutions, known weights of Dowex 50 (0.15 to 0.007 g.) were added, the exchanger (in the ammonium form) having been previously dried in an oven at  $110^\circ\text{C}$  to constant weight, and the capacity having been determined by the column method using lanthanum. A similar batch equilibration method was also used for the barium-hydrogen system.

In the breakthrough volume method a mixture of barium chloride and hydrochloric acid at a total concentration of 0.2 N, or of lanthanum nitrate and ammonium nitrate at a total concentration of 0.1 N, was passed down the column saturated with the univalent ion. Since the multivalent ion is held more strongly, a sharp boundary was obtained and was easily observed by measuring the radioactivity of the liquid leaving the column by means of a Geiger-Müller counter of the liquid flow type.<sup>4</sup> After correcting for the paralysis time of the counting assembly and the decay of the radio-tracer used, the concentration of the barium or lanthanum entering the column was equated to the activity observed after the column was saturated. By measuring the shaded area shown in Fig. 1 the amount of the multivalent ion taken up by the column for a given concentration in solution may be estimated and hence the mass-law product determined.

In order to estimate the mass-law product for values of  $X_{Ba_R}$  and  $X_{La_R}$  below about 0.2 it is necessary to reduce the concentration of the respective ions in solution to  $10^{-5}$  M or less. With such low concentrations very large breakthrough volumes are necessary unless the capacity of the column is kept low. In one experiment a column containing about 10 mg. Dowex 50 of capacity 0.0446 m. equiv. required 1000 ml. of a solution containing  $1.8 \times 10^{-6}$  g./l. of lanthanum nitrate to saturate the column at a value of  $X_{La_R} = 0.417$ . To study the equilibrium at very low concentrations of the multivalent ion it was necessary to use batch methods, since the flow velocity becomes too fast for reasonably sharp boundaries to be obtained if the breakthrough volume is to be reached within a time during which the radio-tracer is still active.

The values of  $X_{Ba_R}$ ,  $X_{Ba_S}$ ,  $C_R$  and  $C_s$  obtained experimentally were substi-



tuted in eqn. (10). Corrections for the activity coefficients  $\gamma_{H^+}^+$  and  $\gamma_{Ba^{2+}}^2$  for mixtures of these two ions are necessary and may be estimated from the activities of the electrolytes in mixtures of barium chloride and hydrochloric acid, for

$$\frac{\gamma_{H^+}^+}{\gamma_{Ba^{2+}}^2} = \frac{\gamma_{\pm HCl}^1 (BaCl_2)}{\gamma_{\pm BaCl_2}^2 (HCl)}, \quad (12)$$

where  $\gamma_{\pm HCl}^1 (BaCl_2)$  and  $\gamma_{\pm BaCl_2}^2 (HCl)$  are the activity coefficients of the electrolytes in mixtures of the two. Now it is possible to estimate  $\gamma_{\pm HCl}^1 (BaCl_2)$  from the data of Randall and Breckenridge<sup>6</sup> by use of the equation

$$\log \gamma_{\pm HCl}^1 (BaCl_2) = \log \gamma_{\pm HCl}^0 + \alpha_{12} c_{BaCl_2},$$

where  $\gamma_{\pm HCl}^0$  is the activity coefficient of hydrochloric acid in solutions of the same ionic strength. From these data plots were made of  $\log \gamma_{\pm HCl}^1 (BaCl_2)$  against  $c_{BaCl_2}$  for solutions of constant ionic strengths, from which  $\alpha_{12}$  was estimated and used to determine  $\gamma_{\pm HCl}^1 (BaCl_2)$  in the solutions.

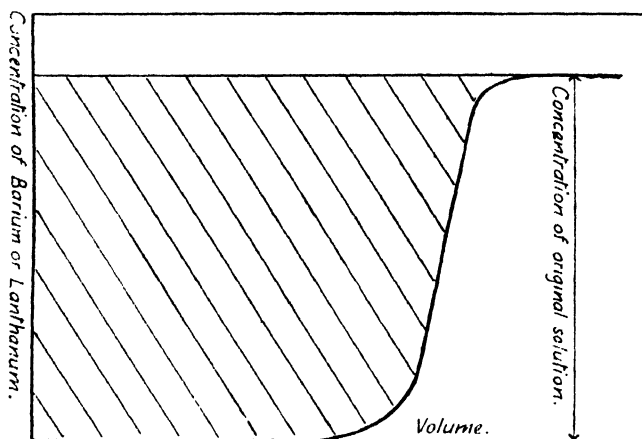


FIG. 1.—Diagrammatic representation of concentration volume plot for breakthrough experiment.

Unfortunately data were not available to enable  $\gamma_{\pm BaCl_2}^2 (HCl)$  to be estimated, but since  $\gamma_{\pm HCl}^1 (BaCl_2)$  was found to differ from  $\gamma_{\pm HCl}^0$  by less than 1 % it was considered reliable to use  $\gamma_{\pm BaCl_2}^0$  as  $\gamma_{\pm BaCl_2}^2 (HCl)$  in solutions of the same ionic strength.

The expression corresponding to eqn. (12) for the lanthanum-ammonium system is

$$\frac{\gamma_{NH_4^+}^1}{\gamma_{La^{3+}}^3} = \frac{\gamma_{\pm NH_4NO_3}^1 (La(NO_3)_3)}{\gamma_{\pm La(NO_3)_3}^3 (NH_4NO_3)} \quad (13)$$

In this case the activity data even for pure lanthanum nitrate are not available and no correction could be made. The results given below therefore do not represent the true value of the mass-law product, but the general variation will be qualitatively correct. The concentration of ammonium nitrate did not vary by more than 0.09 to 0.10 N for  $X_{LaR} < 0.9$  whilst the lanthanum nitrate concentration for the same range was always less than 0.01 N. For values of  $X_{LaR}$  below 0.8 the ammonium ion concentration was 0.099–0.1 N whilst the lanthanum ion concentration was less than 0.001 N. Although the shape of the mass-law product plot may be slightly in error for  $X_{LaR} > 0.8$ , the activity correction is almost certainly constant within about 1 % for lower values of  $X_{LaR}$ .

<sup>6</sup> Randall and Breckenridge, *J. Amer. Chem. Soc.*, 1927, **49**, 1435.

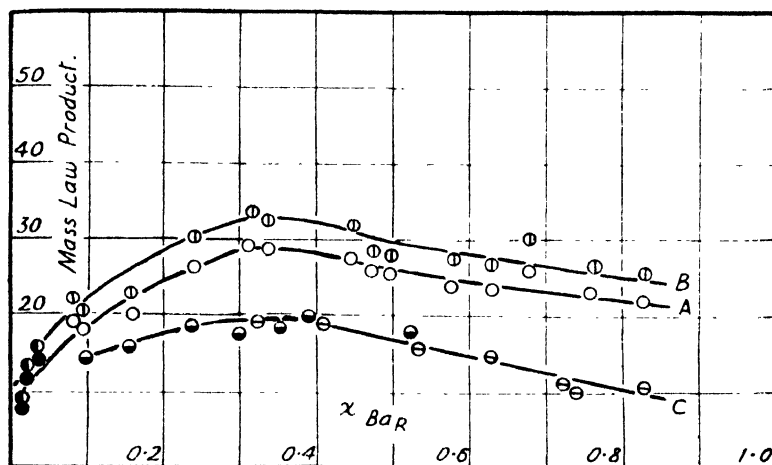


FIG. 2. Mass-law product plot for barium-hydrogen system according to eqn. (10).  
 A.—Room temperature (with no correction for activity coefficients in solution).  
 B.—Room temperature (with activity coefficient correction). C.  $87^{\circ}\text{C}$  (with no correction for activity coefficients in solution).

○ ○ ○ Breakthrough experiments.  
 ● ● ● Batch equilibration experiments.

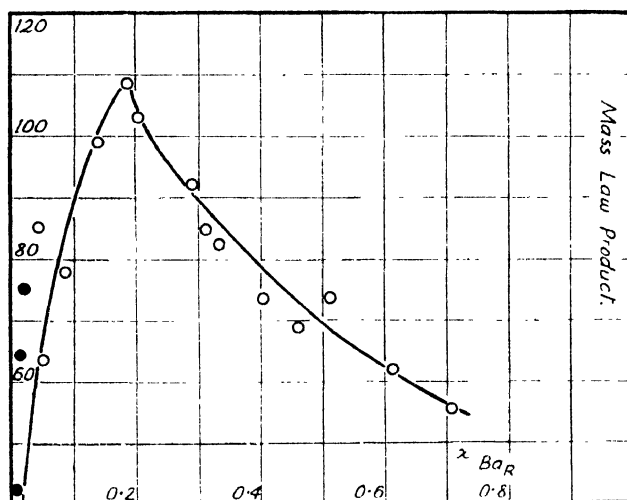


FIG. 3.—Mass-law product plot for barium-hydrogen system at room temperature according to eqn. (3) (with no correction for activity coefficients in solution).

○ Breakthrough experiments.  
 ● Batch equilibration experiments.

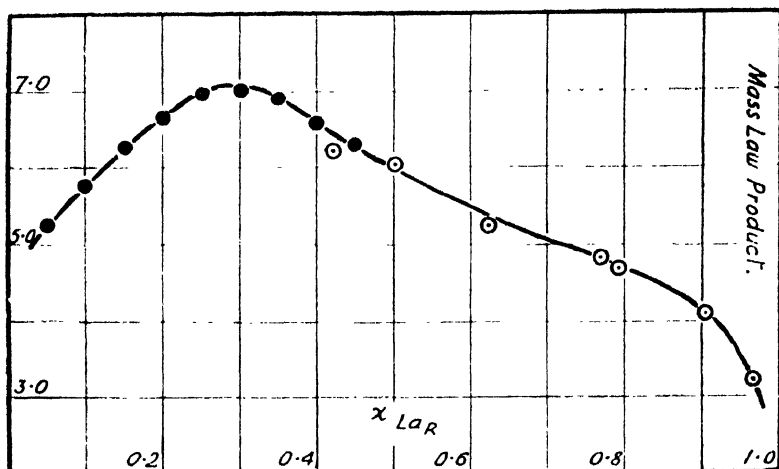


FIG. 4.—Mass-law product plot for lanthanum-ammonium system, according to eqn. (11) (with no correction for activity coefficients in solution).

- Breakthrough experiments.
- Points interpolated from  $X_{LaR}-X_{LaR}$  curve constructed from batch equilibration data.

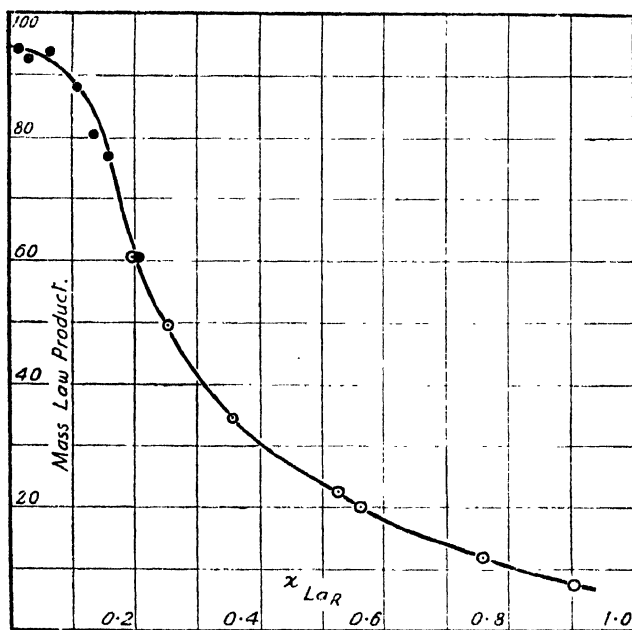


FIG. 5.—Mass-law product plot for lanthanum-ammonium system according to solid solution concept (corresponding to eqn. (3), with no correction for activity coefficients in solution).

- Breakthrough experiments.
- Points interpolated from  $X_{LaR}-X_{LaR}$  curve constructed from batch equilibration data.

## Results and Discussion

The variation in the mass-law product calculated according to eqn. (10) from the equilibrium data given in Table I and II for the barium chloride-hydrochloric acid system is shown in Fig. 2. It will be seen that at room temperature the mass-law product varies by about 50 %, from high values of  $X_{\text{BaR}}$  to a maximum at a value of  $X_{\text{BaR}}=0.31$ . By comparison Fig. 3 shows that the mass-law product derived from the same experimental results by use of eqn. (3) varies by a factor of 2 or more over the same range, the maximum being obtained at  $x_{\text{BaR}}=0.17$  corresponding to  $X_{\text{BaR}}=0.31$ . From this it was concluded that neither approach was strictly valid, although the activities of the ions in the exchanger were more nearly approximated by the use of molar concentrations in the exchanger expressed in arbitrary units. Fig. 2 may be used if desired to estimate the variation of the activity coefficients of the ions in the exchanger, although the values obtained depend on the value of  $K_a$  which is assumed to be valid and on the units of concentration.

For the lanthanum-ammonium system, the equilibrium data are given in Table III, and the curve obtained by use of eqn. (11) without making corrections for the activities of the ions in solution is shown in Fig. 4. The curve calculated assuming the validity of the solid solution concept (corresponding to eqn. (3)) is shown in Fig. 5. Much greater variations of  $K_a$  are obtained, thus supporting the conclusion that eqn. (11) (which assumes

TABLE I  
EXPERIMENTAL DATA FOR  $\text{Ba}^{2+}\text{-H}^+$  SYSTEM (TOTAL CONCENTRATION  
0.2 N)

Total Equivalent Concentration ( $C_s$ )	Equivalent Concentration of Barium ( $C_{\text{Ba}_2}$ )	Capacity of Exchanger (m. equiv.)	No. of m. equiv. of Barium in Exchanger	$\gamma_{\pm \text{HCl}}$ $\gamma_{\pm \text{BaCl}_2}$
<i>Breakthrough Experiments</i>				
0.220	0.01254	0.500	0.414	1.15
0.210	0.0055	0.500	0.380	1.15
0.210	0.00254	0.500	0.340	1.15
0.207	0.00191	0.500	0.315	1.15
0.209	0.00135	0.500	0.289	1.15
0.209	0.000767	0.500	0.248	1.15
0.208	0.000658	0.500	0.237	1.15
0.211	0.000542	0.500	0.224	1.15
0.210	0.000272	0.500	0.168	1.15
0.210	0.000231	0.500	0.156	1.15
0.210	0.000163	0.500	0.121	1.15
0.195	0.0001025	0.130	0.0211	1.14
0.204	0.000063	0.500	0.0481	1.14
0.195	0.0000436	0.130	0.0106	1.14
<i>Batch Equilibration Experiments</i>				
0.200	0.0000263	0.549	0.0201	1.14
0.200	0.0000225	1.360	0.0239	1.14
0.200	0.0000211	0.995	0.0253	1.14

TABLE II

EXPERIMENTAL DATA FOR  $\text{Ba}^{2+}\text{-H}^+$  SYSTEMS AT  $87^\circ \text{C}$  (TOTAL CONCENTRATION 0.2 N)

Total Equivalent Concentration ( $C_s$ )	Equivalent Concentration of Barium ( $C_{\text{Ba}_s}$ )	Capacity of Exchanger (m. equiv.)	No. of m. equiv. of Barium in Exchanger
<i>Breakthrough Experiments</i>			
0.206	0.0201	0.796	0.6573
0.218	0.0105	0.796	0.5890
0.212	0.0080	0.796	0.5760
0.213	0.00320	0.796	0.5029
0.212	0.00159	0.796	0.4260
0.210	0.000629	0.796	0.3258
0.208	0.000365	0.796	0.2560
<i>Batch Equilibration Experiments</i>			
0.206	0.00121	0.432	0.2258
0.206	0.000498	0.427	0.1657
0.206	0.000443	1.719	0.6077
0.206	0.000334	0.832	0.2476
0.206	0.000217	1.292	0.3060
0.206	0.000135	0.859	0.1333
0.206	0.0000812	1.718	0.1672

TABLE III

EXPERIMENTAL DATA FOR  $\text{La}^{3+}\text{-NH}_4^+$  SYSTEM (TOTAL CONCENTRATION 0.1 N)

Total Equivalent Concentration ( $C_s$ )	Equivalent Concentration of Lanthanum ( $C_{\text{La}_s}$ )	Capacity of Exchanger (m. equiv.)	No. of m. equiv. of $\text{La}^{3+}$ in Exchanger
<i>Breakthrough Experiments</i>			
0.1000	0.500	3.107	3.000
0.1002	0.0102	3.107	2.810
0.1000	0.00100	0.410	0.3250
0.0998	0.000676	0.0446	0.03424
0.1000	0.000119	0.0446	0.02778
0.1000	0.0000364	0.0639	0.03208
0.1000	0.0000186	0.0446	0.01860

*Batch Equilibration Experiments* (Typical results from a series of 70 experiments)

0.1000	0.0000797	0.151	0.08800
0.1000	0.0000339	0.0709	0.03470
0.1000	0.0000203	0.0321	0.01400
0.0999	0.00000936	0.0426	0.01485
0.1000	0.00000767	0.0353	0.01110
0.1000	0.00000625	0.0606	0.01800
0.1000	0.00000575	0.0697	0.01950
0.1000	0.00000121	0.0378	0.00375

proportionality between activities and molar concentrations) is more nearly valid.

The fact that variations in the mass-law product are obtained here for systems shown by other authors to give constants is evidently attributable to the fact that most of the earlier mass-law product determinations were made over quite small ranges. For example, Kressman and Kitchener worked within the ranges  $0.76 < X_{\text{BaR}} < 0.88$  and  $0.71 < X_{\text{AlR}} < 0.88$  over which the mass-law product is seen not to vary by more than about 10 %, and Boyd, Schubert and Adamson similarly used restricted ranges (estimated for the barium-sodium system as  $0.64 < X_{\text{BaR}} < 0.91$ , and for the lanthanum-sodium system as  $0.87 < X_{\text{LaR}} < 0.92$ ).

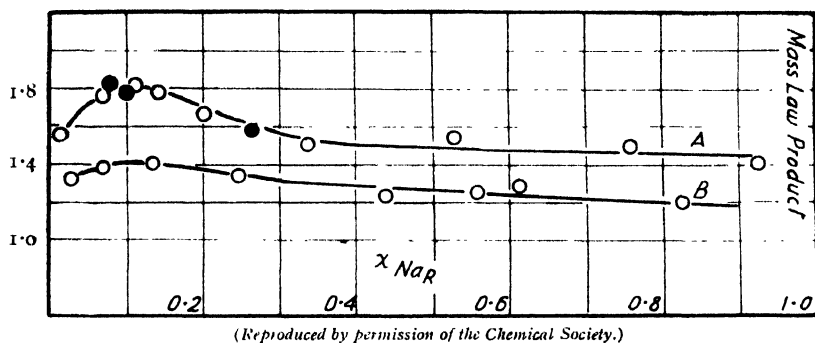


FIG. 6.—Mass-law product for the sodium-hydrogen system ( $C_s = 0.2 \text{ N}$ ).

A.—Room temperature. B.— $-87^\circ \text{C}$  (correction for activity coefficient in solution).

- Breakthrough experiments.
- Batch equilibration experiments.

The use of eqn. (10) and (11) is seen to give results for the exchange of uni-bi and uni-tervalent ions which are very similar to those obtained for uni-univalent exchange. For comparison, the mass-law product of the sodium-hydrogen system<sup>4</sup> at room temperature is shown in Fig. 6. Since the concepts of Kressman and Kitchener and of Bauman and Eichhorn must lead to mass-law products which vary in the same way as those shown in Fig. 2, 4 and 6, no method yet proposed is satisfactory in evaluating the activities of the ions in the exchanger, other than to a first approximation. Whilst it is possible that some other approach may be satisfactory, it seems to us that a fundamental feature may have been omitted in all the methods listed. For instance, whilst it is undoubtedly true that to a first approximation the total capacity of an exchanger is constant, and it is probably correct to assume the *exchange* capacity to be constant, it is possible that the resin takes up non-exchangeable material by adsorption on the surface, by solution in the exchanger or by other processes. In this respect, there is a little experimental evidence; the height of the maximum obtained in the mass-law product plots for the sodium-hydrogen and the barium-hydrogen systems decreases as the temperature increases (see Fig. 2 and 6) as would be expected for the contribution of a non-exchange process (e.g., an adsorption phenomenon) which usually decreases with increasing temperature.

Differences in mass-law product may also be caused by swelling phenomena as has been suggested by Gregor<sup>6</sup> on thermodynamic grounds. It is already

<sup>6</sup> Gregor, *ibid.*, 1948, 70, 1293.

known that ion exchange material swells and contracts in solutions of different electrolytes or in solutions of the same electrolyte in different concentrations and it is possible that swelling may also be different according to the ratio of the ions in the exchanger. Further experimental work is required to ascertain whether such secondary processes, hitherto unconsidered, have an effect on the exchange equilibrium.

We should like to acknowledge the assistance of Mr. P. E. Brown with the experimental work, and Mr. M. A. Hewitt with the computing. We are indebted to Dr. E. Glueckauf for his interest in this investigation and we should like to thank the Director of the Atomic Energy Research Establishment for permission to publish this paper.

ADDENDUM.-- Some confusion seems to have arisen concerning the method of evaluating  $K_a$  by use of eqn. (10) from the experimental results. The figures shown in column 3 of Table I-III were not used as  $C_R$ , as this would lead to a value of  $K_a$  which depends on the amount of exchanger used. In order that  $K_a$  should refer to the same quantity of exchanger,  $C_R$  was taken as the capacity of 1 g. of exchanger (4.3 m. equiv./g.)<sup>4</sup>.

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## EXCHANGE EQUILIBRIA IN ANION-EXCHANGE RESINS: POROUS EXCHANGERS

BY ROBERT KUNIN AND ROBERT J. MYERS

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The exchange equilibria of strong-base and weak-base anion-exchange resins, prepared with various degrees of cross-linking, were examined to determine the effect of this quantity upon the behaviour of the products. By suitable improvements in "porosity" the exchange capacity for large molecules, such as penicillin or coloured bodies in beet molasses, can be increased. The swelling and hydraulic characteristics are also affected by changes in cross-linking. The results are quite analogous to the molecular-sieve effect noted for the adsorption of gases by zeolites and may therefore be considered an "ionic-sieve" effect.

Chromatographic techniques that have employed ion-exchange substances as adsorbents have aided considerably in the analysis of rare earth mixtures,<sup>1</sup> solutions of amino acids,<sup>2</sup> purine bases, nucleic acids<sup>3</sup> and other systems. The principles involved in these separations are similar to those involved in the chromatographic analysis of non-ionic constituents; however, superimposed on these principles are several added ones deriving from the ion-exchange process. Notably successful separations have been achieved through the application of variations in elution agents, degree of column loading, flow-rates during development and particle size of adsorbent. Differences in exchange equilibria have been called into service through searches for adsorbents with specific adsorptive properties, or investigations

<sup>1</sup> Boyd, Schubert and Adamson, *J. Amer. Chem. Soc.*, 1948, **69**, 2818.

<sup>2</sup> Winters and Kunin, *Ind. Eng. Chem.*, 1949, **41**, 460.

<sup>3</sup> Cohn, *Science*, 1949, **109**, 377.

into the effects of complexing agents and of pH on elution. Further extension of the application of exchange equilibria principles in chromatographic analysis has been made through the use of the newer synthetic resin exchange adsorbents that contain various functional groups and possess various degrees of selectivity based upon differences in ionic size.

Equilibria in anion exchangers have been studied extensively by Wiklander,<sup>4</sup> Myers, Eastes and Urquhart,<sup>5</sup> Bhatnagar and co-workers,<sup>6</sup> Griessbach<sup>7</sup> and Kunin and Myers.<sup>8</sup> Most of these studies were conducted on weak-base type anion exchangers, that is, the synthetic resins employed were based upon alkylene polyamines or aromatic diamines. Kunin and McGarvey<sup>9</sup> examined the equilibria relationships in a strong-base or quaternary-type anion exchanger.

Titration curves of anion exchangers with strong acids clearly indicate the strength of the basic group. The order of decreasing exchange ability differs for the weakly basic resins as compared with the strong base chiefly with respect to the position of the hydroxyl ion. In the case of the strongly basic resin the order was found by Kunin and McGarvey<sup>9</sup> to be as follows for the strong-base exchanger, Amberlite IRA-400: citrate > sulphate > oxalate > iodide > nitrate > chromate > bromide > thiocyanate > chloride > formate > hydroxyl > fluoride > acetate. The order of replacing ability is determined by means of "symmetry" studies, in which the percentage exchange effected is plotted against the symmetry value, or milli-equivalents of ion added per milli-equivalent of dry resin. Exchange equilibria relationships such as the Freundlich, Langmuir or Rothmund-Kornfeld equations hold only over certain ranges of concentration, and are best applied when ions of similar size and charge are involved. In general, symmetry plots are readily obtained and are more meaningful in discussions of practical problems in chromatography.

The availability of both weakly and strongly basic anion exchangers offers a range of operations to the laboratory investigator. Such an operating range is applicable mainly in cases of relatively small ions. When larger molecules such as complex colour bodies, penicillin and ascorbic acid are under consideration it is desirable to employ modified exchangers that exhibit greater capacities for large ions. Recent advances in synthetic resin technology have led to the development of ion-exchange resins with different degrees of "porosity" such that larger ions may be adsorbed and eluted effectively. Since the successful application of chromatographic techniques requires operation at or near equilibrium conditions, the porosity of the exchanger becomes a vital factor since rates of diffusion throughout the resin particle vary with both the porosity of the resin and size of the ion.

Samuelson<sup>10</sup> and Bauman<sup>11</sup> have studied the effects of swelling and porosity upon the equilibria of cation exchange in sulphonic acid cation-exchange resins. It is an object of this paper to present some preliminary data on the performance of laboratory preparations of porous anion exchangers of the weak-base and strong-base type.

### Experimental

The resins selected for this study were modifications of the Amberlite IRA-400 (Series A) studied by Kunin and McGarvey<sup>9</sup> in which the density was altered

<sup>4</sup> Wiklander, *Ann. Roy. Agric. Coll., Sweden*, 1946, **14**, 1.

<sup>5</sup> Myers, Eastes and Urquhart, *Ind. Eng. Chem.*, 1941, **33**, 1270.

<sup>6</sup> Bhatnagar *et al.*, *J. Indian Chem. Soc.*, 1941, **18**, 447.

<sup>7</sup> Griessbach, *Z. Ver. Deutsch. Chemiker. Beih.*, 1939, **31**, 1.

<sup>8</sup> Kunin and Myers, *J. Amer. Chem. Soc.*, 1947, **69**, 2874.

<sup>9</sup> Kunin and McGarvey, *Ind. Eng. Chem.*, 1949, **41**, 1265.

<sup>10</sup> Samuelson, *Diss.* (Valhallavägen, Sweden, 1944).

<sup>11</sup> Bauman, *Abstr. Div. Coll. Chem. (Amer. Chem. Soc.)*, 1949, **14**.



TABLE I  
PHYSICAL AND CHEMICAL CHARACTERISTICS OF THE ANION-EXCHANGE RESINS OF SERIES A AND B

## SERIES A

Relative Degree of Cross-linking	Moisture Capacity %	Apparent Density g. (dry)/ml.	Decrease in Resin Volume			Swelling dry→wet %	Total Exch. Capacity m-equiv./g.	Penicillin Capacity m-equiv./g.	Equil. Exch. S = 1 <sup>a</sup>		
			$\mu = 1.0$ %	$\mu = 0.1$ %	$\mu = 0.01$ %				I <sup>-</sup> %	Cl <sup>-</sup> %	F <sup>-</sup> %
1	93	0.15	64	40	26	540	3.2	3.2	66	51	44
2	70	0.19	23	5	1	225	3.1	2.6	76	67	52
3	69	0.26	10	2	0	180	3.1	2.3	80	73	56
4	63	0.32	5	0	0	160	2.9	1.8	81	74	59
8	50	—	4	0	0	125	2.6	0.1	83	76	66

## SERIES B

Relative Degree of Cross-linking	Moisture Capacity %	Apparent Density g. (dry)/ml.	Decrease in Resin Volume			Swelling dry→wet %	Total Exch. Capacity m-equiv./g.	Penicillin Capacity m-equiv./g.	Equil. Exch. S = 1 <sup>a</sup>		
			$\mu = 1.0$ %	$\mu = 0.1$ %	$\mu = 0.01$ %				I <sup>-</sup> %	Cl <sup>-</sup> %	F <sup>-</sup> %
1	80	0.14	64	40	26	540	3.2	3.2	66	51	44
2	69	0.20	23	5	1	225	3.1	2.6	76	67	52
3	61	0.26	10	2	0	180	3.1	2.3	80	73	56
4	55	0.32	5	0	0	160	2.9	1.8	81	74	59
8	45	—	4	0	0	125	2.6	0.1	83	76	66

<sup>a</sup> % exchange on equilibrating the hydroxyl form of the resin with an equivalent amount of the sodium salt of the respective halogen.

by varying the degree of cross-linking, and Series B, a similar set of resins in which the basic groups were of the weak-base type.

The total exchange capacities and exchange equilibria were determined in the manner described by Kunin and Myers<sup>8</sup> and by Kunin and McGarvey.<sup>9</sup> The total capacities of the strong-base resin were also determined by the extent of splitting of sodium chloride. The percentage swelling was determined by a measurement of the difference in volume between two equal weights of resin, one of which was put in the chloride form, the other in the hydroxyl form.

The apparent densities and moisture contents were determined as described previously.<sup>8,9</sup> The capacity for large ions and decolorizing capacity were determined in column experiments. The capacity for decolorizing beet molasses was determined by passing a 15 Brix sugar solution over a 20 ml. bed of resin at a rate of 0.067 ml. per ml./min. The end-point was taken as 20 % colour leakage. The capacity for penicillin was determined by passing dilute solutions (usually about 0.01 N) of the compound over a 2 ml. bed in a 2 ml. pipette at a slow rate, with contact times from three to four minutes. Appropriate chemical tests were employed to detect breakthrough.

The moisture capacity was determined by equilibrating the salt (chloride) form of the resin in water and determining the moisture content of the equilibrated resin.

## Results

**Physical Characteristics.**—The physical characteristics of both resin series are described in Table I in which the variation of moisture capacity, apparent density and swelling characteristics are reported as a function of the degree of resin cross-linking. It is quite obvious that the ability to imbibe moisture and swell decreases markedly upon increasing the degree of cross-linking. Similarly, the degree of resin de-swelling upon electrolyte addition decreases as the degree of cross-linking increases.

**Chemical Characteristics.**—**EXCHANGE CAPACITIES.**—The total capacities of the two resin series are shown in Table I. An increase in the degree of cross-linking is accompanied by a decrease in total exchange capacity for both resin series. However, it is interesting to note that the weak-base resins decrease more rapidly than the corresponding strong bases.

The capacities for penicillin G as determined by a column procedure are shown as a function of degree of cross-linking in Table I. The adsorption of penicillin was studied using 0.01 N solutions of pure penicillin G. Nessler's reagent was used to detect leakage and breakthrough capacity. The much greater capacity for penicillin, in the porous exchangers, is shown clearly by the data. As the degree of cross-linking increases the fraction of the total resin capacity that is available to penicillin decreases markedly. In fact, at high degrees of cross-linking practically no adsorption of the large penicillin anion can be detected. Similar results were obtained with the Series B resins for the adsorption of the weakly acidic coloured bodies in beet molasses.

**EXCHANGE EQUILIBRIA.**—The effect of the degree of cross-linking upon anion-exchange equilibria was determined solely with the Series A, strong-base type resins. The extent of exchange of various halogen anions with the hydroxyl ions of the resin A series is described in Table I. It is quite obvious that the degree of cross-linking has a marked effect upon the exchange equilibria. The results appear to indicate that as the degree of cross-linking increases the equilibrium constant approaches unity.

## Discussion

The results indicate another parameter of importance in chromatography with ionic adsorbents, namely, the degree of porosity of the resin structure in addition to those of pH and basicity or acidity of the adsorbent is to be considered in applications of ion exchangers. By suitable modifications of presently available ion-exchange resins, the exchange capacity for large ions can be increased. Such "porous" exchangers are modified also with respect to swelling and hydraulic characteristics, but certain sacrifices can

be made in these matters to achieve appreciably improved capacities for large anions. Finite limitations on the size and shape of large ions that may be adsorbed are to be expected and will be determined by the structure characteristics of the resin particle. As the degree of cross-linking is increased, the ability to imbibe water and swell is decreased thereby limiting the penetration and exchange capacity towards large ions. These results are quite analogous to the molecular-sieve effect noted by Barrer<sup>12</sup> for the adsorption of gases by the zeolites and may therefore be considered an "ionic-sieve" effect.

The lack of ionic selectivity as the internal pore space increased due to a low degree of cross-linking has been noted by Samuelson<sup>10</sup> and Walton.<sup>13</sup> Walton attributes this effect to the fact that the environment inside of the resin particle tends to approach that of the external solution thereby decreasing the difference between the adsorption of the various ions.

**Conclusions.**—"Porous" modifications of conventional anion-exchange resins have been prepared and examined for their exchange and physical characteristics. It has been found that as the degree of cross-linking decreases the ability to adsorb large anions increases. It has also been noted that decreasing the degree of cross-linking markedly affects the exchange equilibria, decreasing ion selectivity.

The resins examined were synthesized by Dr. Charles McBurney and Dr. Fred Boettner in our Philadelphia laboratories. Miss Ruth Barry and Mr. Frank McGarvey assisted in the determination of exchange capacities.

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Pennsylvania, U.S.A.*

<sup>12</sup> Barrer, *Trans. Faraday Soc.*, 1944, **40**, 206.

<sup>13</sup> Walton, *J. Chem. Educ.*, 1946, **23**, 545.

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## FULLY SWOLLEN ALGINATE GELS AS PERMUTITES: KINETICS OF CALCIUM-SODIUM ION EXCHANGE

BY J. L. MONGAR AND A. WASSERMANN

*Received 20th July, 1949*

On addition of sodium chloride solution to fully swollen cylindrical calcium alginate gels, a stationary sodium chloride concentration in the gel phase is established before appreciable conversion of the calcium alginate into the sodium salt has taken place. These observations, together with the results of other experiments, make it possible to estimate approximate initial values of third-order velocity coefficients characterizing a homogeneous ionic replacement reaction, not a diffusion step.

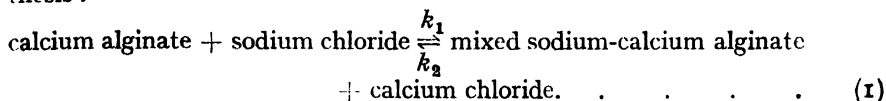
The overall rate of previously investigated ion-exchange reactions appears to depend on the rate of a diffusion stage.<sup>1</sup> Some authors<sup>2</sup> interpreted the results of their kinetic measurements as indicating that the ion exchange itself is the slowest step; but, so far as we are aware, these experiments

<sup>1</sup> See, e.g., Boyd, Adamson and Meyers, *J. Amer. Chem. Soc.*, 1947, **69**, 2836.

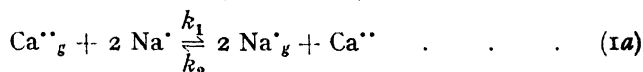
<sup>2</sup> For references, see Duncan and Lister, *Quart. Rev.*, 1948, **2**, 307.

were done with adsorbents of non-uniform shape, and no attempt was made to find out how the velocity coefficients, computed from the analytical data, are influenced by the surface/volume ratio of the exchange material.

The work now to be described deals with the following reversible metathesis:



The chloride ions do not participate stoichiometrically and, therefore, the cation exchange proper can simply be represented by



where the species with the subscript  $g$  are confined to the gel phase, viz., they are "non-permeant," and  $k_1$  and  $k_2$  are third order velocity coefficients relating respectively to the forward and to the reverse process.

The results of the experiments are compatible with the assumption that the initial rate of the forward reaction relates to the homogeneous ion exchange itself, not to a diffusion step, and approximate numerical values of the velocity coefficient  $k_1$  are estimated. The measurements were done with cylindrical alginate gels of high water-content, the surface-volume ratio of the test pieces being varied in the range 34–140 cm.<sup>-1</sup>.

### Results

The alginate gels, containing 92 % of water, were prepared, surface-dried and analyzed by methods described in a paper which we hope to publish in the *J. Chem. Soc.* (referred to below as Part I). Table I shows the numerical values of diffusion-coefficients  $D$  of some simple electrolytes dissolved in these gels.\* These measurements were made by using a method similar to that outlined by Eggleton, Eggleton and Hill,<sup>3</sup> the  $D$  value in the last line being calculated with the help of an equation taken from Barrer.<sup>4</sup>

TABLE I  
DIFFUSION COEFFICIENTS OF SIMPLE ELECTROLYTES IN ALGINATE GELS

Temperature ° C	Gel	Surface/ volume Ratio of Gel (cm. <sup>-1</sup> )	Permeant Electrolyte	Initial conc. of Permeant Electrolyte in Gel (g.-mole per l. Gel)	Diffusion coef. $\times 10^6$ (cm. <sup>2</sup> sec. <sup>-1</sup> )
20 .. ..	Sodium alginate	2	NaOH Na <sub>2</sub> CO <sub>3</sub>	2.30	18 $\pm$ 2
20 .. ..				1.00	8 $\pm$ 2
18 .. ..	Calcium alginate	5	CaCl <sub>2</sub>	0.0500	8.0 $\pm$ 0.8*
0 .. ..		5		to	6.5 $\pm$ 0.7
18 .. ..		60		0.500	7 $\pm$ 1†

\* This value was calculated from observations in which only the earliest stage of the diffusion of calcium chloride from the gel to the solution was taken into account.

† This value relates to the latest stage of the diffusion.

<sup>3</sup> *Proc. Roy. Soc. B*, 1928, **103**, 620.

<sup>4</sup> *Diffusion in and Through Solids* (Cambridge, 1911); cf. also Hill, *Proc. Roy. Soc. B*, 1928, **104**, 39.

\* Sodium alginate is soluble in water, but if the sol is 2 N with respect to sodium hydroxide or carbonate a gradual transition into a gel occurs.

A comparison with the figures listed in *Landolt-Boernstein's Tables* shows that the  $D$  values of  $\text{NaOH}$ ,  $\text{Na}_2\text{CO}_3$  and  $\text{CaCl}_2$  in water are similar to those listed in the last column of Table I; this is of interest because the diffusion coefficients of other simple electrolytes dissolved in a less highly swollen cation exchange material are 5-10 times smaller.<sup>1 5</sup>

In carrying out the kinetic measurements, fully swollen calcium alginate fibres of diameter varying between 0.24 and 1.2 mm. were hung over a hook and rinsed first with water and then with sodium chloride solution of the same temperature, thereby obtaining an effluent  $e_1$ . After known time intervals the flow of the sodium chloride solution was interrupted, the fibre was quickly surface-dried, weighed and rinsed with water until permeant electrolytes were removed from the gel, the effluent thus obtained being designated  $e_2$ . The two effluents were analyzed for calcium, chloride determinations in  $e_2$  being

TABLE II

RESULTS OF EXPERIMENTS, WITH FULLY SWOLLEN CALCIUM ALGinate FIBRES OF 0.48 MM. DIAM. AT 20°, SHOWING THE RAPID ATTAINMENT OF A STATIONARY SODIUM CHLORIDE CONCENTRATION IN THE GEL.

Time of Treatment of Calcium Alginate Fibres with N NaCl Soln. (sec.)	Rate of Flow of NaCl Soln. (cm. <sup>3</sup> /min.)	Weight of Surface-dry Fibre (mg.)	g. equiv. $\times 10^4$		Stationary NaCl concn. in Gel (g.-mole/l. Gel)
			Chloride Released from Fibre*	Calcium Released from Fibre†	
5 .. ..	40	178	1.88	0.03	1.0 $\pm$ 0.1
5 .. ..	18	179	1.4	0.03	0.8 $\pm$ 0.1
10‡ .. ..	40	186	1.8	0.07	1.0 $\pm$ 0.1
10 .. ..	40	180	1.8	0.07	1.0 $\pm$ 0.1
10 .. ..	18	188	1.6	0.07	0.9 $\pm$ 0.1
20 .. ..	40	186	2.1	0.14	1.1 $\pm$ 0.1
30 .. ..	40	192	2.0	0.20	1.0 $\pm$ 0.1
60 .. ..	40	159	2.0	0.35	1.1 $\pm$ 0.1
90 .. ..	40	192	2.3	0.42	1.1 $\pm$ 0.1

\* Into effluent  $e_2$ , during the final rinsing with water.

† Into effluent  $e_1$ , during the treatment with the NaCl solution; 100 % ion exchange corresponds to a release of  $0.72 \times 10^{-4}$  g.-equiv. calcium.

‡ Using the appropriate equation for cylindrical diffusion (see ref.,<sup>4</sup> a diffusion coefficient of  $12 \times 10^{-6}$  cm.<sup>2</sup> sec.<sup>-1</sup> and assuming that the NaCl diffused into the gel is not used up, it can easily be shown that after 10 sec. the NaCl concentration should be about 0.9 g.-mole/l. gel.

TABLE III

KINETICS OF CALCIUM-SODIUM ION EXCHANGE: TYPICAL RESULTS

Temperature  $37 \pm 1^\circ$ ; Initial diam. of fully swollen calcium alginate fibre 1.15 mm.; initial concentration of non-permeant calcium in gel 0.2 g.-mole/l. fully swollen gel; rate of flow of sodium chloride solution 5 cm.<sup>3</sup>/min.; stationary sodium chloride concentration in gel 0.0800 g.-mole/l. fully swollen gel.

Time of treating of fibre with sodium chloride solution (sec.) .. ..	180	300	600	840	1020
% Non-permeant calcium converted into $\text{CaCl}_2$ (which was analyzed in effluent $e_1$ ) .. ..	12	18	27	32	35
$10^4 \times k_1 \times a^2$ (sec. <sup>-1</sup> ) .. ..	6.9	6.6	5.2	4.6	4.2

also carried out; it was possible, therefore, to estimate the sodium chloride concentration in the alginate gel and to establish that the quantity of calcium chloride in  $e_2$  was negligibly small. The results of typical measurements are in Table II and III. The figures in the last line of Table III were obtained by integration of the following rate equation,

$$dx/dt = k_1 a^2 (b - x), \quad (2)$$

where  $a$  is the stationary sodium chloride concentration in the gel,  $b$  and  $b - x$  are the concentration values of the non-permeant calcium respectively at zero time and time  $t$ . The latter concentration could be estimated by taking into account the figures in the second line of Table III and the results of control tests (Part I) which showed that no significant storage of  $\text{CaCl}_2$  in the fibre occurs, that there is no membrane hydrolysis, and that the ratio

$\frac{\text{No. of g.-equiv. non-permeant calcium removed from gel as result of ion exchange}}{\text{No. of g.-equiv. non-permeant sodium incorporated into gel}}$   
is unity.

TABLE IV  
KINETICS OF CALCIUM-SODIUM ION EXCHANGE: SUMMARY OF RESULTS

Run No.	Temp. °C	Initial Diam. Fibre* (mm.)	Stationary NaCl conc. in Gel† (g.-mole/l. Gel)	Rate of Flow of NaCl Soln. (cm. <sup>3</sup> /min.)	pH of Soln.	$k_1^2$ (l. <sup>2</sup> g.-mole <sup>-2</sup> sec. <sup>-1</sup> )
1		0.28	1.10	2		0.020
2		0.43	1.00	2		0.014
3		0.55	1.00	2		0.016
4		0.55	1.00	10		0.014
5		0.55	1.00	20		0.014
6		0.96	0.800	2		0.016
7		0.28	0.800	2		0.016
8	20	0.40	0.400	2		0.030
9		0.28	0.130	2		0.14
10		0.45	0.100	2	5-6	0.16
11		0.45	0.100	10-20		0.16
12		0.50	0.100	40-45		0.12
13		1.1	0.08	5		0.14
14		1.1	0.08	30		0.18
15		0.28	0.07	2		0.16
16	5	0.55	0.10	40		0.16
17	5	1.1	0.08	5		0.12
18	30	1.0	0.08	5		0.16
19	37	1.1	0.08	5		0.13
20†	20	0.45	0.10	45	10	0.12
21†	20	1.1	0.08	5	10	0.12

\* The diameter  $2r$  of all fibres was much smaller than their length; the surface/volume ratio is therefore  $2/r$ .

† Each figure listed in this column was obtained from the result of tests similar to those indicated by the data in Table II.

‡ In these experiments the calcium alginate was rinsed with a sodium chloride solution containing carbonate-free sodium hydroxide.

Graphical extrapolation of  $k_1 a^2$  to small conversion ratios leads to an initial value of  $8.5 \pm 0.5 \times 10^{-4}$  sec.<sup>-1</sup>, corresponding to an initial velocity coefficient of  $k_1^2 = 0.13 \pm 0.04$  l.<sup>2</sup> g.-mole<sup>-2</sup> sec.<sup>-1</sup>. Similar experiments were carried out under conditions indicated by the figures in column 2-6 of Table IV, the relevant initial velocity coefficients being listed in the last column.

The accuracy of these  $k_1^2$  values is  $\pm 30\%$  in most runs, but in a few experiments, e.g., No. 15, it is  $\pm 45\%$ . Such errors are considerably larger than

those of most published rate measurements relating to one-phase systems or to heterogeneous reactions involving less highly swollen adsorbents. It should also be noted that the fibre diameter could only be varied within a rather narrow range.

### Discussion

The kinetic significance of velocity coefficients deduced from eqn. (2) is based on the validity of the following assumptions: (i) the diffusion of NaCl from the outside solution into the gel is fast compared to the rate of formation of  $\text{CaCl}_2$ ; (ii) the volume of the alginate gel remains constant throughout the course of observation; (iii) the diffusion of  $\text{CaCl}_2$  from the gel to the outside solution is fast compared to the rate of the reverse reaction (1). The figures in Table II and the results of similar tests show that a stationary NaCl concentration in the gel is rapidly attained before an appreciable conversion of calcium alginate has occurred; and it appears, therefore, that as a first approximation (i) is valid. It is known,<sup>6</sup> on the other hand, that the conversion of calcium alginate into the mixed salt is accompanied by considerable swelling, so that the second assumption is not compatible with the actual facts. The swelling of the alginate gels must give rise to a decrease of  $b - x$  in eqn. (2), which in turn should bring about an increase of  $k_1 a^2$ . The figures in the last line of Table III,

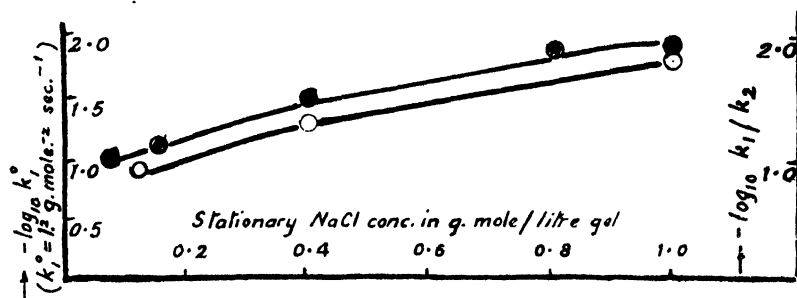


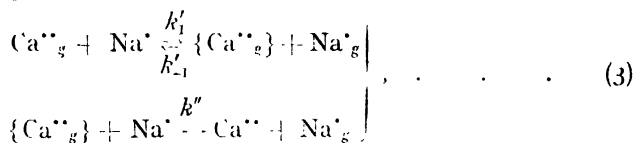
FIG. 1. — Concentration dependence of velocity and equilibrium coefficients. Temperature 20°; (A) black circles left ordinate; (B) open circles right ordinate.

and the results of other experiments, show, however, that the  $k_1 a^2$  values decrease with increasing conversion, and we believe that under these conditions the influence of the back reaction over-compensates the effect due to the swelling. The instantaneous rate of the reverse reaction (1), which is probably proportional to the square of the concentration of the non-permeant sodium ions, must decrease rapidly with decreasing conversion into the mixed alginate, and it is reasonable, therefore, to postulate the validity of (iii) for the initial stage of the interaction between calcium alginate and sodium chloride. Such a hypothesis is in accordance with experiments showing that the  $k_1'$  values are not detectably dependent on the rate of flow of the NaCl solution or on the fibre diameter (cf. Runs Nos. 1-7 and 12-16 of Table IV). If it should be possible in future to carry out more accurate measurements it may be found that the initial velocity coefficients increase somewhat with increasing surface/volume ratio of the test piece. Observations of this kind would not invalidate any essential conclusions to be derived from this work; but the correct initial velocity coefficients would have to be estimated by plotting the fibre diameter,  $r$ , against  $k_1^{(r)}$  and by extrapolating to  $r = 0$ .

<sup>6</sup> Mongar and Wassermann, *J. Physiol.*, 1947, **106**, 32P. MacArthur, Mongar and Wassermann, *Nature*, 1949, **164**, 110.

The  $k_1^0$  values in Table IV increase with decreasing NaCl concentration, as shown by curve A in the Figure. The trend can be taken to be a solvent effect, the dielectric constant and other properties of a medium being dependent on the salt concentration. Curve B represents the concentration dependence of the equilibrium coefficient  $k_1/k_2$ , the later values having been determined by measurements done in a static system (Part I). These graphs provide confirmatory evidence for the correct interpretation of the kinetic measurements, it being well known that in many cases an alteration of chemical conditions gives rise to similar changes of rate and equilibrium coefficients.<sup>7</sup>

The association of certain simple electrolytes involves covalent forces<sup>8</sup> and it is not impossible that similar effects play a role, to a certain extent, in the salts of alginic acid. Moreover, the solvation of permeant sodium or calcium ions is probably not identical with that of these cations if they are stoichiometrically combined with the alginate residues, thereby being held in proximity of a highly charged poly-anion. The ion exchange may be accompanied, therefore, by an alteration of both covalent and solvation forces and this should give rise to an appreciable activation energy or to a small probability of reaction. The observed temperature dependence of the velocity coefficient  $k_1'$  (see Runs Nos. 16-19 in Table IV) is not compatible with an overall activation energy exceeding a few kcal. It has to be taken into account, however, that the calcium-sodium ion exchange, like other homogeneous ionic processes<sup>9</sup> of the stoichiometric type 3 reactants  $\rightarrow$  product, can take place as a result of consecutive bimolecular processes. These can be formulated in the present case as follows:



where  $\{\text{Ca}^{++}_g\}$  is the symbol for an intermediate in which the non-permeant calcium is combined with only one carboxylate grouping of an alginate residue;  $k'_1$ ,  $k'_{-1}$  and  $k''$  are bimolecular velocity coefficients and the significance of the other symbols is the same as in (1a). It can be shown, with the help of the stationary state approximation, that  $k'_1$  is proportional to  $k''k'_1/k'_{-1}$ , provided  $k'_{-1}[\text{Na}^+_g] > k''[\text{Na}^+]$ . The overall activation energy  $E$  of the forward reaction (1) would be given, therefore, by  $E = E'' + E'_1 - E'_{-1}$ , where the various terms on the right-hand side relate to the corresponding velocity coefficient in (3). Thus a small overall activation energy does not necessarily imply a small activation energy of elementary bimolecular replacement steps.

The work described in this paper forms part of an investigation into the influence of ion exchange on the molecular shape of chain-like polyelectrolytes. A grant from the Department of Scientific and Industrial Research is gratefully acknowledged.

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<sup>7</sup> For a theoretical treatment of such correlations see, e.g., Evans and Polanyi, *Trans. Faraday Soc.*, 1935, **31**, 492.

<sup>8</sup> Bell and Prue, *J. Chem. Soc.*, 1949, 362, where references to previous work will be found.  
E.g., Weiss, *J. Chem. Soc.*, 1944, 309.



# SOME METHODS FOR EXTENDING THE SCOPE OF PARTITION CHROMATOGRAPHY

BY ALFRED A. LEVI

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Three methods are described for extending partition chromatography to substances with limited solubilities in water.

Although in a large majority of cases adsorption chromatography is a very convenient and efficient process, partition chromatography offers certain advantages, especially for colourless substances and for quantitative work. Thus :

(i) Partition chromatography as described by Martin and Synge<sup>1</sup> offers scope for the separation of acids, since few adsorbents have been found which will give satisfactory chromatograms with acidic substances.

(ii) Substances left as bands on the column after a partition chromatogram are always easily eluted by simple extraction with more of the stationary phase. The quantitative elution of adsorbed material is sometimes difficult, or impossible.

(iii) Conditions on a partition chromatogram are generally easily reproducible. This is not always the case with adsorption, since it is difficult to make successive batches of adsorbents with reproducible properties.

(iv) Distribution \* isotherms for partition are conveniently measured in tap funnels, or on columns. Hence the data required for calculations based on chromatographic theory are easily obtained.

(v) Experience has shown that with silica gel as support little or no "coning" occurs in carefully prepared columns, and the fronts of the bands are substantially horizontal even in quite large columns.

On the other hand, the original method of partition chromatography is applicable only to the comparatively few substances which have at least a moderate solubility in water. The present paper reviews some methods by which this limitation can be to some extent overcome.

In the first method water is replaced by another solvent immiscible or only partially miscible with the flowing phase. This method has given some excellent separations, but is again severely limited by the relatively small number of solvent pairs which can be used. Examples are : (i) For the separation of isomeric hexachlorocyclohexanes<sup>2</sup> : stationary phase, nitromethane ; flowing phase, *n*-hexane. (ii) For the separation of fatty acids<sup>3</sup> : stationary phase, methanol ; flowing phase, low-boiling liquid paraffins.

In the few examples so far published, isotherms for substances separated appear to be linear or nearly so. This method should therefore be worth

<sup>1</sup> Martin and Synge, *Biochem. J.*, 1941, **35**, 1358.

<sup>2</sup> Ramsay and Patterson, *J. Ass. Off. Agric. Chem.*, 1946, **29**, 337.

<sup>3</sup> Ramsay and Patterson, *J. Ass. Off. Agric. Chem.*, 1948, **31**, 139, 164.

\* It is suggested that the terms "distribution ratio, distribution isotherm," etc., be used to cover all cases where a substance distributes itself between two phases, whether by adsorption, partition, ion exchange, or other mechanism.

considering whenever a mixture with suitable physical properties is encountered.

In the second method, water as stationary phase is replaced by a solution of some substance which reacts reversibly with some or all of the constituents of a mixture. The most generally useful system consists of a solution of a suitable buffer as stationary phase. An immiscible solvent forms the flowing phase. By this means mixtures of acids or bases can be separated. This technique, which requires no unusual practical procedure, offers the following advantages :

(i) By the choice of a suitable solute in the stationary phase a partition chromatographic technique becomes available for substances which have a relatively low solubility in water.

(ii) Another variable for the control of the rate of movement of the bands becomes available in the case of acids and bases.

(iii) Extremes of acidity and alkalinity can be avoided when handling sensitive material.

(iv) By the use of concentrated solutions as stationary phase relatively large quantities of material can be separated.

(v) Impurities in the support, such as traces of alkali or foreign metals in silica gel, become of less importance in the presence of the buffer.

This technique was successful in the separation of the different penicillins,<sup>4</sup> and has also given some useful results in the analysis of alkaloid mixtures.<sup>5</sup> It suffers from two disadvantages :

(i) With colourless substances, indicators on the column can no longer be used, as in the Martin and Synge technique. This means that observations must be largely confined to the effluent solution. There are now a number of procedures available for this and in view of the flexibility of the system, this is no great disadvantage.

(ii) In general the distribution of substances between buffer and solvent seems to follow isotherms of the Freundlich type, viz.,  $Q = aC^b$ . The bands obtained are unsymmetrical and with large loads much overlapping of bands may occur. It has been shown that the position and shape of a band of phenylacetic acid on such a column with ether as solvent can be predicted from chromatographic theory, and from the isotherms determined in tap funnels.<sup>5</sup>

The difficulty of "tailing" was serious with the penicillins, and in all cases chromatographic column fractionation has had to be supplemented by crystallization to obtain homogeneous material.

It is worth pointing out that curved isotherms are by no means confined to buffered solutions. Many instances are known where substances are distributed between solvent and water in conformity with Freundlich distribution isotherms and in some cases the value of the exponent is quite low (ca. 0.5). This difficulty can be turned to advantage by making use of the technique of displacement development,<sup>6</sup> since for this procedure to be successful, distribution ratio must vary with concentration, i.e., the isotherms must be curved. The study of displacement development on partition columns in these laboratories has brought to light some points of interest not previously discussed, and these are described below.

For partition chromatography isotherms can be determined by three methods :

(i) By simple partition experiments in tap funnels.

(ii) By passing sufficient of a solution of known concentration  $C_0$  through

<sup>4</sup> Levi *et al.*, *Biochem. J.*, 1948, **43**, 257, 262.

<sup>5</sup> Evans and Partridge, *Quart. J. Pharm. Pharmacol.*, 1948, **21**, 126 ; **31**, 441.

<sup>6</sup> Tiselius, *Arkiv. Kemi, Min. Geol. A*, 1943, **16**, 1.

a column to give an incompletely developed band. From measurements of the volumes of effluent and of the concentration variation of the tail, the isotherm can be calculated. For the Freundlich isotherm it is only necessary to find the retention volume  $V$  of the front of the constant concentration zone, and the volume  $v$  at which the constant concentration zone just finishes. If  $Q_0$  is the concentration in the stationary phase,  $V_0$  the initial volume  $S$  the pore volume of the column, and  $P$  the weight of stationary phase, then :

$$\begin{aligned} Q_0/C_0 - aC_0^{n-1} &= (V - S)/P, \\ dQ_0/dC_0 &= anC_0^{n-1} = (v - V_0 - S)/P, \\ n &= (v - V_0 - S)/(V - S). \end{aligned}$$

In practice  $v$  is liable to be somewhat too low, with a corresponding effect on  $n$ , but the procedure is convenient, and sufficiently accurate for such purposes as choice of developer for a displacement chromatogram.

(iii) By determination of specific retention volumes at a series of known initial concentrations. This is the most accurate method and for partition is convenient, if some device is available for detecting the issuing zones.

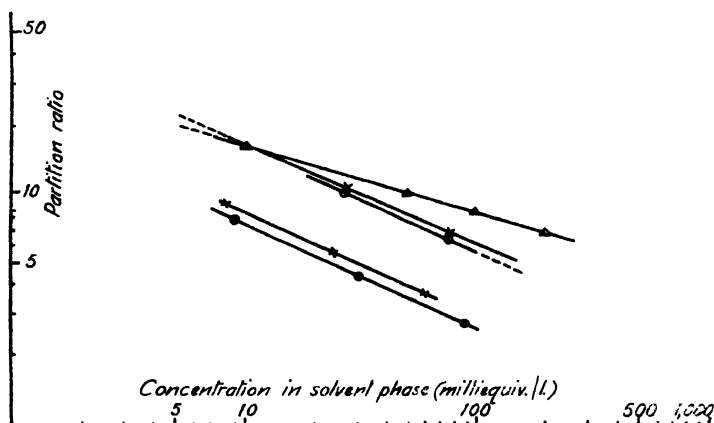


FIG. 1.—Series of distribution isotherms determined on partition chromatograms by method (iii).

From the family of isotherms so obtained a developer at suitable concentration is chosen, and the characteristic step concentrations are read off from the graphs (see Fig. 1), or are calculated. Thus for the Freundlich isotherm if  $C_D$  is the developer concentration, and  $C_1, C_2, \dots$  the final step concentrations of the components,

$$Q_1/C_1 = Q_2/C_2 = \dots = Q_D/C_D,$$

$$\text{or } \log a_1 + (n_1 - 1) \log C_1 = \log a_2 + (n_2 - 1) \log C_2 = \dots = \log a_D + (n_D - 1) \log C_D.$$

The following interesting deduction can be made from this equation. If we assume for simplicity that  $n$  is the same for all the substances, we have

$$\log C_2 - \log C_1 = (\log a_1 - \log a_2)/(n - 1).$$

Since  $n$  usually has values lying between 0.5 and 1.0 it is seen that concentration differences between steps may be quite large for very small differences in the values of  $a$ . In fact, the only limit to the resolving power of this technique is the sensitivity to small changes in concentration of the device used to follow the issuing zones.

If an approximate knowledge of the composition of the mixture to be analyzed is available the length of column occupied by the completed displacement chromatogram can be calculated. This is useful as a guide to the amount of charge which can be put on a given column. Experience has shown that the column should be some three to four times the above calculated length. If the front of the developer moves a distance  $x_D$  when unit volume passes through the column, and there are  $m_1, m_2, \dots$  milli-equivalents of the components present in the initial charge, then the length of column occupied by the completed chromatogram will be

$$x_D \left[ \frac{m_1}{C_1} + \frac{m_2}{C_2} + \dots \right].$$

If from a previous frontal analysis, or by other means,  $x_0$ , the distance travelled per unit volume by the front of the original mixed zone before development commences is known, then the volume of developer required to complete the displacement chromatogram can be calculated.

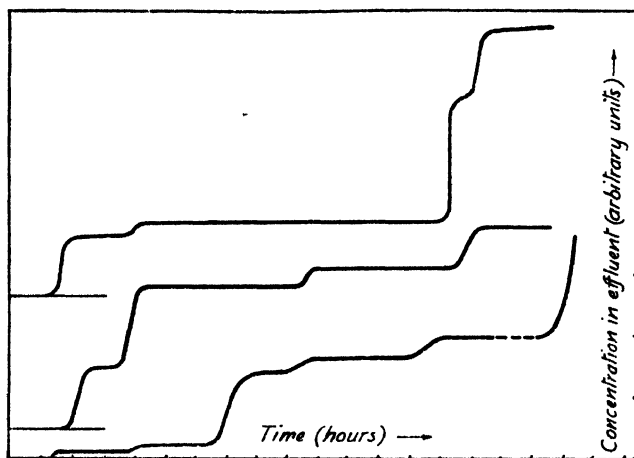


FIG. 2.—Tracings of partition chromatograms developed by the displacement technique.

Thus, assume that until displacement is complete the front of the whole system continues to move at a rate  $x_0$  per unit volume. When displacement is completed the front of the new zones will have moved the same distance. Hence  $x_0 (V_0 + V_D) = x_D V_D + x_D [m_1/C_1 + m_2/C_2 + \dots]$ , where  $V_0$  is the volume of the original solution.

Therefore 
$$V_D = \frac{x_D (m_1/C_1 + m_2/C_2 + \dots) - V_0 x_0}{x_0 - x_D}.$$

Since in most cases the front of the undisplaced zones will move a distance less than  $x_0 V_D$  this expression will give a maximum value for  $V_D$ .

From what has been said above, it can be seen that this technique offers very great scope for the quantitative analysis of complex mixtures of closely related materials (e.g., homologous series, position isomerides, and stereo-isomerides). By working on a small scale with narrow columns the technique of coupled filters, or "front straighteners"<sup>7</sup> has not been found necessary in partition chromatograms (see Fig. 2). These would doubtless

<sup>7</sup> Claesson, *Arkiv. Kemi, Min. Geol. A*, 1947, **24**, 1.

be advantageous on a preparative scale, but this possibility has not yet been adequately explored.

The great sensitivity of the above method of displacement development to small differences in partition characteristics involves some restriction on the range of substances which can be separated under any given set of conditions. Hence for a very crude mixture some preliminary separation is desirable. For this purpose partition chromatography offers another device in the use of a stationary phase consisting of a solution or suspension of some substance which reacts irreversibly with the components of a mixture.<sup>8</sup> The chromatogram which results from passing a mixture through such a column resembles a frontal analysis in that a number of zones are formed equal to the number of substances present. Each new zone from the bottom to the top of the column contains a new component (in addition to those already present) in the order of increasing affinity for the stationary phase. The picture differs from frontal analysis in that the total molecular concentration in each step is the same (except in so far as it may be modified by physical partition between solvent and water alone). Separation cannot be complete in such a system, and the method is of little quantitative value. The products, being in solution, are readily recovered from the silica or other support by simple extraction with water. This procedure was valuable in the case of crude penicillin, especially for the earlier samples before the fermentation process was under adequate control. Large quantities of material could be considerably improved in quality without serious loss by treatment in ether solution on silica gel columns impregnated with caustic soda solution. A partial separation of bases (e.g., a crude extract of alkaloids) could obviously be similarly effected.

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<sup>8</sup> Catch, Cook and Heilbron, *Nature*, 1942, **150**, 163.

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## PARTITION CHROMATOGRAPHY ON PAPER WITH SPECIAL REFERENCE TO QUANTITATIVE SEPARATIONS

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Recent improvements in the method of partition chromatography on paper are reviewed. By modification of the solvents used new groups of substances can be separated. In some cases (e.g., esters of the fatty acids) reversed phase chromatograms are most convenient. Many attempts have also been made to employ the paper chromatogram for quantitative estimations; considerable success has been achieved in the case of the amino acids which, after elution from the paper, can be converted into Cu salts suitable for colorimetric determination. Some of the factors making for low recoveries, such as the impurities present in the filter paper, are also discussed.

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The method of partition chromatography on paper is at present being developed in two different directions. New groups of substances are being brought within the scope of the method by the introduction of new solvent mixtures and modified techniques for the detection of the spots on the

chromatogram. In addition, efforts are being made to obtain quantitative recoveries of those materials whose separation is already well established. No attempt will be made here to list all the groups of substances to which the method has already been applied, its scope has already been reviewed.<sup>1 2 3</sup> The purpose of the present work is to consider, first, the conditions necessary for successful separations on paper chromatograms and, second, some of the various techniques which have been used for the quantitative estimation of the separated substances. For separations to take place the following factors need to be considered.

(a) **Choice of Solvent.**—A solvent must be found partially miscible with water giving sufficiently different partition coefficients for the various substances of the mixture to be separated. In practice this can be taken to mean differences of at least 10 %. Where no single solvent is available giving adequate differences of partition coefficient for all the components of the mixture, a second solvent can be subsequently utilized by means of the two-dimensional technique.<sup>4</sup> Although many substances can be separated in systems of this kind in which the solvent held stationary in the filter paper is water, for substances of a very non-polar character, it may be necessary to work in non-aqueous systems. Thus Boldingh<sup>5</sup> by pre-treating filter paper with latex has been able to separate esters of fatty acids by means of methanol. In this system the rubber itself acts as the immobile solvent. Boldingh has also successfully used mixed organic solvents such as methanol, benzene and 1/1 methanol acetone on rubber paper. He remarks that the rubber acts as a carrier for the less polar solvent. This is no doubt true, but as using methanol acetone the  $R_f$  values are considerably increased compared with those obtained with methanol alone, the effect of the acetone in the mobile phase must also be important.

The use of aqueous systems employing mixed organic solvents as the moving phase has often proved necessary. Also the partition coefficients of the substances under separation have been modified by suitable additions to the aqueous phase. Systems giving convenient partition coefficients may be obtained in one of the following ways.

(i) The  $R_f$  values can be changed by the admixture of a suitable proportion of a second organic solvent. This solvent may be completely miscible with water. So long as not more than enough is used for the formation of two phases, useful results may be expected. The effect of the addition of a more polar solvent is, of course, to increase the water content of the organic solvent phase and thus the  $R_f$  values of all types of substance. Results of this kind were obtained by Partridge<sup>6</sup> who measured the  $R_f$  values of the sugars in *n*-butanol and in *n*-butanol containing 10 % ethanol. Before the introduction of partition chromatography on paper this principle was used by Martin and Synge<sup>7 8</sup> when they increased the  $R$  values of the acetyl amino acids on silica gel chromatograms by employing progressively higher concentrations of *n*-butanol in chloroform. A second effect of the alcohol in this system was its virtual elimination of adsorption of the acetyl amino acids by the gel. Even though most substances seem scarcely to be adsorbed by filter paper, in those cases, e.g., the chromatography

<sup>1</sup> Consden, *Nature*, 1948, **162**, 359.

<sup>2</sup> Hais, *Chem. Listy*, 1948, **42**, 125.

<sup>3</sup> Hais and Rábek, *Chem. Listy*, 1949, **43**, 80.

<sup>4</sup> Consden, Gordon and Martin, *Biochem. J.*, 1944, **38**, 224.

<sup>5</sup> Boldingh, *Experientia*, 1948, **4**, 270.

<sup>6</sup> Partridge, *Biochem. J.*, 1948, **42**, 238.

<sup>7</sup> Martin and Synge, *Biochem. J.*, 1941, **35**, 1358.

<sup>8</sup> Gordon, Martin and Synge, *Biochem. J.*, 1943, **37**, 79.

of inorganic ions,<sup>9</sup> where such effects may be significant they are no doubt reduced by the admixture of more polar organic solvents.

(ii) If the substances being separated are acids or bases their  $R_f$  values can be greatly influenced by adjustment of the pH of the aqueous phase. As an example, the effect of ammonia on chromatograms developed with phenol in decreasing the  $R_f$  values of the dicarboxylic and increasing those of the basic amino acids may be mentioned. Conversely, acetic acid has been used by Lugg<sup>10</sup> to assist the separation of certain organic acids by *n*-butanol. Buffer solutions<sup>11</sup> have also been used in a rather similar way both on filter paper and on silica gel chromatograms for the separation of the penicillins. A further advantage of the addition of buffers, at least on silica gel chromatograms, is that partition chromatograms can be run by displacement development and thus much larger amounts can be handled.

In addition, the  $R_f$  values of certain compounds can be modified by the addition of substances mainly soluble in the organic solvent phase. Thus Winsten and Eigen<sup>12</sup> have found that the  $R_f$  values in *n*-butanol of the various members of the streptomycin group could be markedly increased by the addition of 2 % of *p*-toluenesulphonic acid. The solvent also contained 2 % of piperidine which served partially to de-ionize the basic groups of the streptomycins. The amount of *p*-toluenesulphonic acid used was such that the pH of the system (determined after the addition of an equal volume of water) was on the alkaline side. However, it was noted that separations could still be obtained if so much *p*-toluenesulphonic acid had been used that the pH was acid. It is interesting to compare the effect of *p*-toluenesulphonic acid with that of ammonia in *n*-butanol. Thus if it is assumed that the increased  $R_f$  values of the streptomycins were not solely due to the piperidine, then *p*-toluenesulphonic acid, although an acid substance, must have affected the system in the same sense as would be expected for the addition of ammonia. It seems likely that additions of this type have their effect both because they increase the polarity of the organic solvent phase and because as acids they specifically modify the ability of this phase to dissolve the substances being separated. That the effect is not due only to the former cause seems to be indicated by the observation<sup>13</sup> that the addition of *p*-toluenesulphonic acid to collidine increases the  $R_f$  values of the basic preferentially to those of the neutral amino acids.

(b) **Adsorption by the Paper.**—If the separations are to depend only on partition effects, the substances in the mixture to be separated must not be adsorbed by the paper. Fortunately few substances are strongly adsorbed by untreated filter paper. Perhaps the largest group of substances for which adsorption is likely to be important are the organic dyestuffs. Attempts to separate dyes by partition chromatography on paper often lead to resolution of the mixture. However, closer examination of the system may reveal that partition effects have been secondary (as is the case if the separation can be carried out equally well with the dry solvent). Adsorption chromatography on strips of filter paper of the streptomycins using a 2 % aqueous solution of ammonium chloride as solvent has been described by Horne and Pollard.<sup>14</sup> Unfortunately no resolution of the different streptomycins was obtained.

<sup>9</sup> Arden, Burstall, Davies, Lewis and Linstead, *Nature*, 1948, **162**, 691.

<sup>10</sup> Lugg and Overell, *Nature*, 1947, **160**, 87.

<sup>11</sup> Goodall and Levi, *Nature*, 1946, **158**, 675.

<sup>12</sup> Winsten and Eigen, *J. Amer. Chem. Soc.*, 1948, **70**, 3333.

<sup>13</sup> Gordon (unpublished).

<sup>14</sup> Horne and Pollard, *J. Bact.*, 1948, **55**, 231.

Among large molecules the proteins are also known to be adsorbed, at least from salt solutions, by filter paper. These effects will not be discussed further here as they may be the subject of another contribution. The examples mentioned may be enough to illustrate the possibility of adsorption in paper chromatography. This possibility should not automatically lead to the opposite view that the separations in all systems employing solvents which do not form two phases with water must necessarily be due to adsorption. As was first suggested to the author by Dr. Synge,<sup>15</sup> a system employing, for instance, pyridine and water can be considered to be a partition chromatogram because the effect of the cellulose hydroxyl groups results in the existence of a very different milieu inside the fibre from that of the moving phase.

(c) **Chemical Reactivity of Solvents and Paper.**—The substances under separation must not react either with the paper or with the solvents used. For the amino acids and many of the other substances for whose separation paper chromatography has proved applicable, these criteria have proved to be rather easily fulfilled. However, the recent finding of Moore and Stein,<sup>16</sup> that using 1/2/1-*n*-butanol-*n*-propanol-0.1 N HCl on starch chromatograms 6% and 7% of aspartic and glutamic acids respectively became esterified, suggests that closer and more quantitative examination of paper chromatograms in which mixtures of acids and alcohols are used might reveal similar effects. Cellulose itself fortunately shows little or no reactivity towards either the substances separable on paper chromatograms or the solvents used for their separation. The problem thus becomes that of the possible reactions which may occur between the substances under separation, the solvents and impurities present in the paper. This will be further discussed in the next section.

(d) **Techniques for Detection and Estimation of Spots.**—Finally for qualitative work a suitable detection technique must be available. In practice the sensitivity, reliability and ease of application of such techniques may be the decisive factor in determining whether paper chromatography is to be used. Certainly the remarkable sensitivity of ninhydrin by which less than 1  $\mu$ g. of an amino acid can just be made visible has been most useful. The small scale of the method as a whole, e.g., 1–20  $\mu$ g. per component of the mixture under separation for the amino acids, the sugars and for the purines and pyrimidines, has the advantage that it is usually possible to develop as many chromatograms as there are different detection techniques available. Particularly when dealing with complex biological fluids this type of approach by which several classes of compounds can be simultaneously investigated may be of value.

If quantitative estimations are to be attempted, not only must the substances be located, but sufficiently accurate means must be found for their estimation either *in situ* or after removal from the paper. For quantitative purposes, obviously the most suitable detection techniques are those which do not permanently affect the substances to be estimated. The most generally applicable means by which this can be accomplished is certainly the examination of the spots in u.-v. light. In this way spots of purines, pyrimidines, amino acids and peptides can be shown up. Holiday<sup>17</sup> has reported that as little as 0.5–1  $\mu$ g. per cm.<sup>2</sup> of the purines or pyrimidines can be detected if u.-v. light of wavelengths between 230 and 400 m $\mu$  is used, the most important wavelengths being in the region

<sup>15</sup> Synge (personal communication).

<sup>16</sup> Moore and Stein, *J. Biol. Chem.*, 1949, **178**, 53.

<sup>17</sup> Holiday and Johnson, *Nature*, 1949, **163**, 216.



of 254 m $\mu$ . Although only amounts of more than 3  $\mu$ g. per cm.<sup>2</sup> of the amino acids can be detected<sup>18</sup> the use of this technique has been continued by Woiod.<sup>19</sup> By this means it is possible to avoid the necessity of developing parallel guide chromatograms and also the most inconvenient process of visualizing the spots as their mercury derivatives in the case of the purines and pyrimidines.<sup>20</sup>

The only chemical method so far reported which ultimately leaves the substances unchanged on the chromatogram seems to be that of Brante.<sup>21</sup> This author has sprayed chromatograms of bases such as amino-ethanol, choline and creatine with an alcoholic solution of iodine. Brown spots are formed which disappear on being allowed to stand. Unfortunately this reagent is not very sensitive for the amino acids, but, as noted by Brante, it may prove useful in quantitative experiments.

Turning now to the various methods which have been used for the quantitative estimation of substances separated by paper chromatography, these may be divided into those in which the substances are estimated on the paper, usually as coloured derivatives, and those in which they are first eluted and then estimated in solution. The former approach seems only to have been employed for the coloured substances formed from the amino acids by ninhydrin and for substances containing radio elements. Thus several authors<sup>22 23</sup> have reported that useful rough estimations can be made by visually comparing the spot strengths with those of a series of standard spots. Attempts have also been made to increase the accuracy of this method by the preliminary division of the amino acids into acidic, neutral and basic types.<sup>24</sup> A spectrophotometer was used for estimating the strengths of the spots but as yet no indication has been given of the accuracy thus obtained. A spectrophotometer has been similarly used by Bull<sup>25</sup> who has been at pains to standardize the conditions of colour development. Under the conditions chosen, arginine, serine, valine, glutamic acid, leucine, threonine, alanine and lysine gave the same amount of colour per mole, the probable error for a single determination being almost 9 %. This result is somewhat unexpected as Moore and Stein,<sup>26</sup> who have carefully measured the amounts of colour formed in solution in presence of a reducing agent, have reported, for instance, that threonine forms only 81 % as much colour as does lysine. It may possibly be the case that some of the amino acids form more nearly equivalent amounts of colour in solution than on paper. On the other hand Bull<sup>25</sup> found that glycine and methionine give less colour on paper than does leucine, whereas Moore and Stein<sup>26</sup> indicate that under their conditions these three amino acids yield almost identical amounts of colour per mole. Possibly more thorough investigations of the optimum conditions for the development of the ninhydrin colour on paper may lead to some reduction in the rather large errors at present involved in this technique. Work of this kind may also explain why Pratt and Auclair<sup>27</sup> find that glycine and glutamic acid are the amino acids giving visible spots at minimum strength, whereas Moore and Stein<sup>26</sup> have found lysine to give the strongest colour in solution.

Where substances containing radioactive isotopes have been separated

<sup>18</sup> Phillips, *Nature*, 1948, **161**, 153.

<sup>19</sup> Woiod, *Biochem. J.* (in press).

<sup>20</sup> Vischer and Chargaff, *J. Biol. Chem.*, 1948, **176**, 703.

<sup>21</sup> Brante, *Nature*, 1949, **163**, 651.

<sup>22</sup> Consden, Gordon, Martin and Synge, *Biochem. J.*, 1947, **41**, 596.

<sup>23</sup> Polson, *Biochim. Biophys. Acta*, 1948, **2**, 575.

<sup>24</sup> Block, *Science*, 1948, **108**, 608.

<sup>25</sup> Bull, Hahn and Baptist, *J. Amer. Chem. Soc.*, 1949, **71**, 550.

<sup>26</sup> Moore and Stein, *J. Biol. Chem.*, 1948, **176**, 367.

<sup>27</sup> Pratt and Auclair, *Science*, 1948, **108**, 213.

the detection technique, i.e., the exposure of successive areas of the chromatogram to the counter, is itself suitable as a quantitative estimation. An apparatus designed for the estimation of spots containing  $P^{32}$  has recently been described.<sup>28</sup> In order to estimate the amino acids by means of radioactive isotopes conditions have been worked out for their quantitative conversion into *p*-iodophenylsulphonyl (pipsyl) derivatives containing  $I^{132}$ . After chromatography on paper nearly quantitative recoveries were obtained for the few amino acids tested.<sup>29</sup> Further work on this promising method will be awaited with interest.

Although techniques in which the substances are eluted from the paper prior to estimation cannot have the simplicity of those already mentioned, they would seem to offer several compensating advantages. Thus, once the substance is in solution it can be treated with a much wider variety of reagents than while it is still on paper. This should, for instance, allow better control of the conditions under which the ninhydrin colour is formed from the amino acids. Unfortunately, no attempt has been reported to utilize the valuable information on this subject already obtained by Moore and Stein.<sup>26</sup>

The most successful attempts to estimate quantitatively the amino acids involve their conversion into copper complexes. Thus Martin and Mittelmann<sup>30</sup> used what was essentially the method of Pope and Stevens,<sup>31</sup> in which the amino acids are allowed to react with a suspension of copper phosphate in phosphate-borate buffer. After centrifugation from the excess of copper phosphate, the amount of copper in solution was estimated polarographically. No attempt was made to determine the recoveries of the amino acids after elution from the paper, but, since the current increased linearly with the amount used, estimations were shown to be possible if several comparison chromatograms of known amounts of each amino acid were developed simultaneously with each analysis. The results in a test analysis of gramicidin S clearly substantiated the theory that this peptide consists of five different amino acids in equimolar proportions. Only in the case of one of these acids, leucine, did the amount found deviate more than 5 % from that expected.

The copper method has also been used by Woiwod,<sup>19</sup> who has modified the reagent by replacing sodium borate with di-sodium hydrogen phosphate. He has thus obtained a more stable suspension, lower blanks, and a linear relationship between amino nitrogen and copper. The copper has been estimated colorimetrically with sodium diethyldithiocarbamate instead of polarographically. In order to obtain sufficient accuracy for those amino acids present as the weaker components of mixtures, the analyses have been conducted on larger amounts (1–50  $\mu$ g. amino nitrogen) than those employed by Martin and Mittelmann.<sup>30</sup> In this way similar accuracies seem to have been achieved, and, because of the convenience of colorimetry compared with polarography, this is probably at the moment the method of choice. Generally Woiwod, like Martin and Mittelmann, has employed the comparative method, but in addition he has measured the losses sustained as an amino acid moves down the chromatogram. Thus only 86 % of the original amount of glycine could be recovered after the spot had moved 10 in. down a chromatogram developed with 40 % *n*-butanol–10 % acetic acid–50 % water. In partial explanation of such low recoveries, Woiwod mentions an interesting observation to the effect that fluorescent

<sup>28</sup> Lindberg and Hummel, *Arkiv. Kemi*, 1949, **1**, 17.

<sup>29</sup> Keston, Udenfriend and Levy, *J. Amer. Chem. Soc.*, 1947, **69**, 3151.

<sup>30</sup> Martin and Mittelmann, *Biochem. J.*, 1948, **43**, 353.

<sup>31</sup> Pope and Stevens, *Biochem. J.*, 1939, **33**, 1070.

material can often be seen extending right down to the front of the solvent. He considers that this may be due to failure of the amino acid to reach equilibrium with the mobile phase. However, as the usual treatment with ninhydrin, which is believed to be capable of revealing smaller amounts of amino acids than are visible in u.-v. light, does not show up such streaks, it seems more likely that the amino acid must have been converted into a form no longer capable of giving a colour with ninhydrin. The existence of some substances in certain filter papers capable of combining with the amino acids is already known from the observations of Consden *et al.*<sup>4</sup> on the formation of copper complexes of the amino acids. These are progressively formed as the amino acids move down the chromatogram. These authors<sup>32</sup> have also noted the formation on paper chromatograms of a zinc salt of cysteic acid which like the copper complexes gives a pink colour with ninhydrin. The  $R_f$  values of these metal complexes are close to those of the corresponding free amino acids, but there seems no reason why there may not be other impurities in the paper which could combine to form complexes moving with any  $R_f$  value. The more the  $R_f$  values of such impurity amino-acid complexes differ from that of the amino acid itself, the more difficult it must be to obtain quantitative recoveries from the paper. The solution of these difficulties may either be similar to that adopted by Consden *et al.*<sup>4</sup> who added a reagent to the paper which prevented the action of the copper by itself combining with it; or else it may be necessary to employ special purification of the paper. If purification of the natural paper making materials proves inadequate, use may in time be made of papers formed entirely from synthetic fibres.

These findings naturally raise the question of the occurrence of similar losses as other substances move down paper chromatograms. Fortunately, very accurate methods of analysis exist both for the purines and pyrimidines and for the sugars after elution from the filter paper. For the purines and pyrimidines it is only necessary to measure their absorption at the appropriate wavelength in u.-v. light. Both Vischer and Chargaff<sup>20</sup> and Hotchkiss<sup>33</sup> have used this method without finding necessary a comparative approach similar to that employed for the amino acids. They report accuracies of  $\pm 4\%$  and do not mention progressive losses. The figures quoted suggest slight losses for those spots which have moved further down the chromatograms but are hardly sufficient to be decisive. The need for further work on this question is apparent.

Even more accurate analyses of sugars after separation on paper chromatograms have been reported,<sup>34</sup> and again no mention of progressive losses has been made. It may not be accidental that materials similar to the matrix of the chromatogram can be recovered with least loss.

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<sup>32</sup> Consden, Gordon and Martin, *Biochem. J.*, 1946, **40**, 580.

<sup>33</sup> Hotchkiss, *J. Biol. Chem.*, 1948, **175**, 315.

<sup>34</sup> Flood, Hirst and Jones, *J. Chem. Soc.*, 1948, 1679.

# SEPARATIONS USING ZEOLITIC MATERIALS

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A short account has been given of aspects of zeolitic sorption which are important in separating molecular mixtures. Some natural crystalline zeolites fall into three classes of molecular-sieve sorbent each capable of separating mixtures by selective occlusion if there are sufficient differences in shape and dimensions between the molecules in the mixture. The molecules removed by occlusion must always be comparatively small, but separations may be quantitative after a single exposure to the zeolite.

By cation interchange and by burning out interstitial ammonium ions a diversity of modified molecular-sieve sorbents can be produced with extension of the classification as molecular sieves into numerous classes, the potentialities of which as selectively sorbing media have not yet been fully investigated. Moreover, by operating at low temperatures major differences may develop in the sorption rates of gases all of which are rapidly sorbed at higher temperatures, and in which there are only small differences in molecular dimensions.

Zeolites have sometimes proved very suitable for resolving molecular mixtures.<sup>1 2</sup> Complete separations can often be effected, but to use the crystals effectively one must be acquainted with their sorbent and molecular-sieve properties. In the present summary, factors determining these properties will be described, for natural and artificially modified zeolites.

**Some General Properties of Gas-sorbing Zeolites.**—Crystalline zeolites act as sorbents only when the water normally present in interstices within the crystals has been removed by heat and evacuation. Not all these minerals are, however, capable of taking up gases and vapours in place of the interstitial water. There are three structural types of importance from the viewpoint of the sorptive property:—

Bonding in two dimensions weaker than that in the third. (Fibrous zeolites.)	Scolecite Natrolite Edingtonite Thomsonite
Bonding in layers stronger than that between layers. (Laminar zeolites.)	Heulandite Stilbite
Bonding strong in all dimensions. (Robust three-dimensional network zeolites.)	Chabazite Gmelinite Mordenite Analcite Harmotome Levynite

Fibrous and laminar zeolites tend to shrink when interstitial water is removed, and this shrinkage may be irreversible if the heating is too severe. If the heat treatment during outgassing is more gentle, water may be resorbed, or its place may sometimes be taken by the small polar molecule  $\text{NH}_3$ . However, these minerals do not occlude non-polar gases, and have no general power of sorption. Robust network zeolites, on the other hand, may occlude both polar and non-polar gases copiously, although even here

<sup>1</sup> Barrer, *J. Soc. Chem. Ind.*, 1945, **64**, 130 and 133; *Brit. Pat.* 548,905; *U.S. Pat.* 2,306,610.

<sup>2</sup> Barrer and Belchetz, *J. Soc. Chem. Ind.*, 1945, **64**, 131.

there is a wide variety in the accessibility of the interstitial volume. Harmotome and, as a rule, analcite sorb only small polar gases; mordenite and levynite sorb many small molecules, and gmelinite and chabazite occlude a still greater variety of sorbates.<sup>3 4 5 6</sup>

No zeolite has hitherto been discovered which will occlude really large molecules, and although a number still remain to be investigated it is unlikely that any crystalline zeolite will prove capable of doing so. In order for this to be the case the aluminosilicate framework would have to be so open that instability would almost certainly ensue, with collapse of the whole structure into a crystal more economical of space and so of higher density. Nevertheless the chabazite lattice, based on a comparatively open aluminosilicate framework, is surprisingly stable, and at least one purely synthetic zeolite has been made by hydrothermal methods with a stable framework just as open as that of chabazite.<sup>7 8</sup> This zeolite has no naturally occurring counterpart, and there remains the possibility that other synthetic open framework crystals may be grown, with the limitation on the degree of openness noted above. As a group the gas-sorbing zeolites are therefore capable of sorbing only smaller molecules, and their potential value in chromatography is for removing such smaller molecules from admixture with larger ones.

**Molecular-Sieve Properties of Zeolites.**—Several researches have been directed towards establishing the molecular-sieve behaviour of some zeolites and also towards the artificial modification of this behaviour.<sup>1 2 8 9 10</sup> The molecular-sieve properties to be described refer to thoroughly outgassed finely powdered zeolites, but with an outgassing temperature inside the range of thermal stability of the crystals. Variable outgassing conditions can strongly influence the molecular-sieve behaviour.

Several sorbate molecules of fairly accurately known shape and dimensions were used to determine the accessibility of the crystalline interstices as sorption sites. *iso*Butane, propane, ethane, methane, argon, nitrogen and oxygen were used for this purpose, and three categories of molecular-sieve crystal were characterized:

Class I  
(Chabazite, gmelinite,  
synthetic zeolite BaAlSi<sub>2</sub>O<sub>8</sub>.*n*H<sub>2</sub>O).

Do not sorb *iso*-C<sub>4</sub>H<sub>10</sub> (and other *iso*-paraffins). Sorb C<sub>3</sub>H<sub>8</sub> (and other *n*-paraffins) slowly at room temperature or above, and sorb C<sub>2</sub>H<sub>6</sub>, CH<sub>4</sub> and molecules of smaller cross-section rapidly.

Class II  
(Mordenite).

Do not sorb *n*- or *iso*-paraffins. Sorb C<sub>2</sub>H<sub>6</sub> and CH<sub>4</sub> slowly and N<sub>2</sub> and molecules of smaller cross-section rapidly at room temperature or above.

Class III  
(Ca- and Ba-mordenites).

Do not sorb *n*- or *iso*-paraffins. Negligible sorption of C<sub>2</sub>H<sub>6</sub> and CH<sub>4</sub>. Rapid sorption of N<sub>2</sub>, O<sub>2</sub> and molecules of smaller cross-section at room temperature or above.

<sup>3</sup> Barrer, *Proc. Roy. Soc. A*, 1938, **167**, 392.

<sup>4</sup> Barrer, *Trans. Faraday Soc.*, 1944, **40**, 555.

<sup>5</sup> Barrer and Ibbitson, 1944, **40**, 195 and 206.

<sup>6</sup> Barrer, *Ann. Reports*, 1944, 31.

<sup>7</sup> Barrer, *J. Chem. Soc.*, 1948, 127.

<sup>8</sup> Barrer and Riley, *ibid.*, 1948, 133.

<sup>9</sup> Barrer, *Trans. Faraday Soc.*, 1949, **45**, 358.

<sup>10</sup> Barrer, *Nature* (in press).

It was demonstrated that the *shape* rather than the molecular *volume* of the sorbate exercises the decisive influence upon the sorptive behaviour. The cross-section referred to in the above classification of several zeolites is that normal to the direction of the greatest length of the molecule. For all *n*-paraffins this cross-section is the same in their most extended configuration. Since chabazite, for example, slowly occludes the standard molecule propane, it should slowly occlude all *n*-paraffins. This was verified using *n*-butane, *n*-pentane and *n*-heptane. Increasing the chain length exerted a secondary influence in further slowing down the velocity of intracrystalline diffusion, but as sorption equilibrium was approached large amounts of all the *n*-paraffins studied were occluded. *n*-Heptane has a considerably larger molecular volume but a smaller cross-section than *isobutane* which is completely excluded from the intracrystalline sorption sites.

Again, the standard molecule *isobutane*,  $\text{CH}(\text{CH}_3)_3$  is as noted not sorbed by chabazite; and accordingly other molecules of similar shape and size such as  $\text{CHCl}_3$  and  $\text{CHBr}_3$  are also not sorbed. On the other hand the molecules  $\text{ClCH}_2\text{Cl}$ ,  $\text{ClCH}_2\text{CH}_3$ ,  $\text{BrCH}_2\text{CH}_3$  and  $\text{BrCH}_2\text{Br}$  which have similar shapes and dimensions to  $\text{CH}_3\text{CH}_2\text{CH}_3$  are like propane slowly occluded by chabazite. Many other examples of predictable behaviour were observed and the sorptive action towards a variety of species is summarized in Table I.

**Selective Sorption from Mixtures.**—The molecular-sieve property of zeolites would suggest that clear-cut separations could be obtained in molecular mixtures of which one constituent was freely occluded by the zeolite, while the other constituent, having the wrong molecular dimensions, was not occluded. Proof of this simple principle does not appear to have been published before 1945, but since then the possibility of such separations has been abundantly verified.<sup>1,2,8</sup> Two methods of verification were employed. In one—the static method—a quiescent gas or liquid mixture was brought into contact with the outgassed zeolite, and after an interval of time the non-sorbed gas or liquid was removed and its composition investigated.\* The second procedure—the percolation or chromatographic method—consisted in passing a gaseous or liquid mixture through a column of outgassed zeolite, and investigating the composition of the effluent. This method which was employed only in one or two instances (with  $\text{C}_2\text{H}_6$ – $\text{C}_3\text{H}_8$  and  $\text{CH}_2\text{Cl}_2$ – $\text{CHCl}_3$  mixtures) can undoubtedly be extended, and chromatograms might be developed for suitable molecular mixtures.

The actual separations carried out have been given elsewhere<sup>1,2,6,8</sup> and we may therefore only make some general comments upon them. Class I molecular-sieve sorbents (chabazite, and synthetic  $\text{BaAlSi}_2\text{O}_6 \cdot n\text{H}_2\text{O}$ ) can be used to separate *n*-paraffins of lower molecular weights from all *iso*-paraffins, aromatic hydrocarbons or naphthenes. In these same sorbents methane derivatives, in which the substituents are small groups such as  $-\text{NH}_2$ ,  $-\text{OH}$ ,  $-\text{CH}_3$ ,  $-\text{CN}$ ,  $-\text{Cl}$ , are rapidly occluded, and similarly substituted ethanes are slowly occluded. Quantitative separations of these species are usually possible from compounds which, like *iso*-paraffins or aromatics, are excluded from the sorption sites (e.g., column 4, Table I).

In mordenite, a Class II sorbent, methane derivatives in which the substituents are as before,  $-\text{NH}_2$ ,  $-\text{OH}$ ,  $-\text{CH}_3$ ,  $-\text{CN}$ ,  $-\text{Cl}$ , are slowly occluded, but the similarly substituted ethanes are not sorbed, so that separations were possible of substituted methanes from the corresponding substituted ethanes, as well as from many other species as given in Table I. In the

\* The time-intervals ranged from an hour to more than a week and the temperature from 20° to 250° C according to the occlusion velocity of the sorbable component, and its thermal stability.

TABLE I  
MOLECULES OCCLUDED OR EXCLUDED BY THREE CLASSES OF MOLECULAR SIEVE

	Typical molecules rapidly occluded at room temperature or below	Typical molecules moderately rapidly or slowly occluded at room temperature or above	Typical molecules which are not appreciably occluded at room temperature or above
Section (i) Class I minerals	He, Ne, A  H <sub>2</sub> , N <sub>2</sub> , O <sub>2</sub> CO, CO <sub>2</sub> COS, CS <sub>2</sub> H <sub>2</sub> O HCl, HBr NO NH <sub>3</sub> CH <sub>3</sub> OH CH <sub>3</sub> NH <sub>2</sub> CH <sub>3</sub> CN HCN Cl <sub>2</sub> CH <sub>3</sub> Cl, CH <sub>3</sub> Br CH <sub>3</sub> F CH <sub>2</sub> Cl <sub>2</sub> , CH <sub>2</sub> F <sub>2</sub> CH <sub>4</sub> , C <sub>2</sub> H <sub>6</sub> C <sub>2</sub> H <sub>2</sub> CH <sub>2</sub> O H <sub>2</sub> S CH <sub>3</sub> SH	C <sub>3</sub> H <sub>8</sub> and simple higher <i>n</i> -paraffins C <sub>2</sub> H <sub>5</sub> OH C <sub>2</sub> H <sub>5</sub> NH <sub>2</sub> C <sub>2</sub> H <sub>5</sub> F, C <sub>2</sub> H <sub>5</sub> Cl, C <sub>2</sub> H <sub>5</sub> Br I <sub>2</sub> , HI CH <sub>3</sub> Br <sub>2</sub> CH <sub>3</sub> I C <sub>2</sub> H <sub>5</sub> CN  C <sub>2</sub> H <sub>5</sub> SH  H.COOCH <sub>3</sub> , HCOOC <sub>2</sub> H <sub>5</sub> CH <sub>3</sub> COCH <sub>3</sub>  CH <sub>3</sub> CO.OCH <sub>3</sub> NH(CH <sub>3</sub> ) <sub>2</sub> , NH(C <sub>2</sub> H <sub>5</sub> ) <sub>2</sub>	Aromatic hydrocarbons cyclo and <i>iso</i> -paraffins. Derivatives of these hydrocarbons. Heterocyclic compounds (e.g., thiophene, pyrrole, pyridine). CHCl <sub>3</sub> , CCl <sub>4</sub> , CHCl = CCl <sub>2</sub> , CH <sub>2</sub> CHCl <sub>2</sub> , CHCl <sub>2</sub> CCl <sub>3</sub> , C <sub>2</sub> Cl <sub>6</sub> , and analogous bromo and iodo compounds. Secondary straight chain alcohols, thiols, nitriles and halides. Primary amines with NH <sub>2</sub> group attached to a secondary carbon atom. Tertiary amines. Branched chain ethers, thio-ethers and secondary amines
Section (ii) Class II minerals	He, Ne, A H <sub>2</sub> , O <sub>2</sub> , N <sub>2</sub> CO NH <sub>3</sub> H <sub>2</sub> O	CH <sub>4</sub> , C <sub>2</sub> H <sub>6</sub> CH <sub>3</sub> OH CH <sub>3</sub> NH <sub>2</sub> CH <sub>3</sub> CN CH <sub>3</sub> Cl, CH <sub>3</sub> F HCN Cl <sub>2</sub>	All classes of molecules in columns 3 and 4 of section (i)
Section (iii) Class III minerals	He, Ne H <sub>2</sub> , O <sub>2</sub> , N <sub>2</sub> H <sub>2</sub> O	A HCl NH <sub>3</sub>	All molecules referred to in column 4, section (ii). Also CH <sub>4</sub> , C <sub>2</sub> H <sub>6</sub> , CH <sub>3</sub> OH, CN <sub>2</sub> NH <sub>2</sub> , CH <sub>3</sub> SH, CH <sub>3</sub> CN, CH <sub>3</sub> Cl, CH <sub>3</sub> F

case of Class III sorbents the separations effected were of small polar inorganic molecules (H<sub>2</sub>O, NH<sub>3</sub>, HCl) from organic compounds.

Some partial or complete separations were also effected using zeolites in which both components were sorbed but at different velocities. Using chabazite these included the pairs C<sub>2</sub>H<sub>6</sub>-C<sub>3</sub>H<sub>8</sub>, CH<sub>3</sub>OH-C<sub>2</sub>H<sub>5</sub>Br, C<sub>2</sub>H<sub>5</sub>OH-*n*-C<sub>7</sub>H<sub>16</sub>, C<sub>2</sub>H<sub>5</sub>OH-CH<sub>2</sub>Br<sub>2</sub>, CH<sub>3</sub>CN-CH<sub>2</sub>Br<sub>2</sub>, the molecule first mentioned being occluded most rapidly. A number of separations were also carried out which could not be effected by direct distillation, either because the mixtures were azeotropic pairs (H<sub>2</sub>O-C<sub>2</sub>H<sub>5</sub>OH, CH<sub>3</sub>OH-CH<sub>3</sub>COCH<sub>3</sub>, H<sub>2</sub>O-dioxane, CS<sub>2</sub>-CH<sub>3</sub>COCH<sub>3</sub>, C<sub>2</sub>H<sub>5</sub>OH-*n*-C<sub>7</sub>H<sub>16</sub>, C<sub>2</sub>H<sub>5</sub>OH-toluene) or because the constituents had nearly the same boiling points (*n*-heptane-*isooctane*).

**Artificial Modification of Molecular-Sieve Sorbents.**—Water, which is strongly sorbed by zeolites, by occupying sorption sites which would otherwise be accessible to other sorbates, can greatly diminish not only the

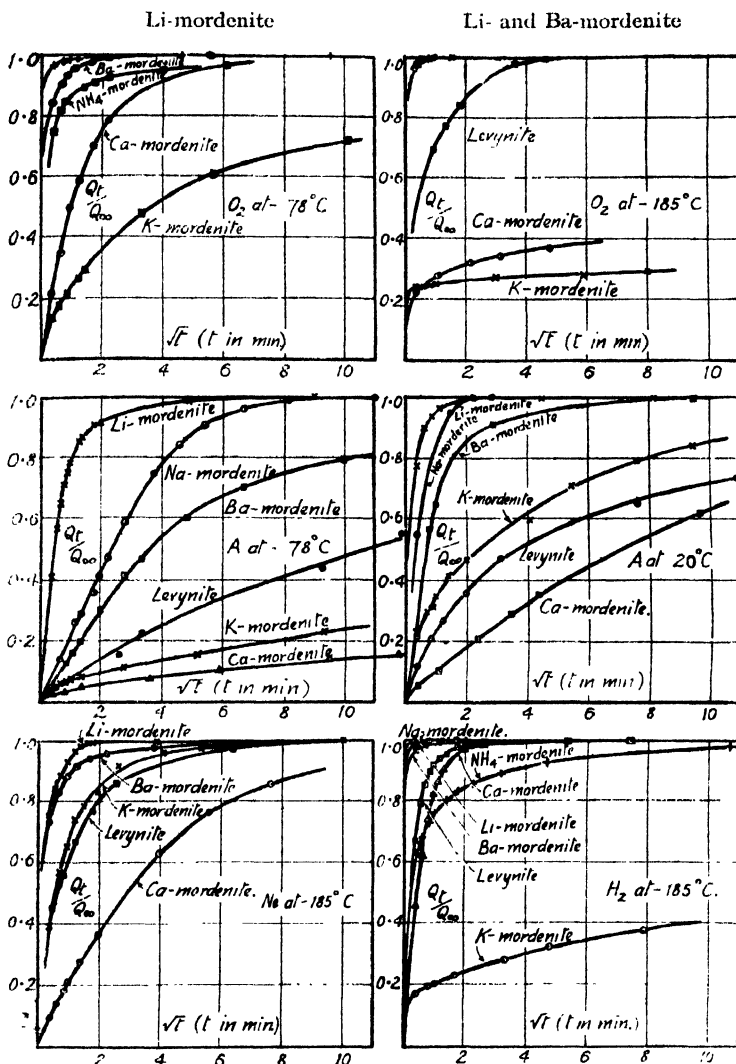


FIG. 1.—Relative sorption rates of each of several sorbates in levynite and in a series of mordenites modified by base exchange. A great measure of control over the sorption rate can be exercised by a suitable choice of base-exchange mordenite.

amount but also the velocity of sorption and the molecular-sieve character of the crystals. This behaviour has been studied in any detail only with chabazite,<sup>11 12</sup> which was progressively transformed from a Class I sorbent into one whose properties more nearly recalled a Class III sorbent. Although a small amount of water might be a useful modifying agent, larger amounts by depressing the sorptive capacity of the zeolite unduly would have a poisoning effect. Indeed, other strongly occluded polar molecules sometimes appear to act as poisons and limit separations of mixtures which in their

<sup>11</sup> Lamb and Woodhouse, *J. Amer. Chem. Soc.*, 1936, **58**, 2037.

<sup>12</sup> Emmett and DeWitt, *ibid.*, 1943, **65**, 1253.



absence could easily be resolved. In resolving mixtures of *n*- and *iso*-paraffins, for instance, the gases should be free of moisture or other small polar molecules capable of preferential sorption and so of acting as poisons.

Other methods of modifying the zeolites have now been evolved which are free from objections. The first of these is by cation interchange.<sup>9, 13</sup> In many zeolites exchange occurs freely although higher temperatures are usually needed than is the case with amorphous or gel zeolites such as Permutit. Thus exchange is best carried out by hydrothermal methods\* in the temperature range 150°–250° C using an excess of the exchanging salt. In this way, for instance, the ions in chabazite have been largely replaced by Ca, Sr, Ba, NH<sub>4</sub>, K, Cs, Na and Li; and similarly mordenites enriched in Ca, Ba, NH<sub>4</sub>, K, Na and Li have been prepared. In analcite exchange is more specific, the replacements  $\text{Na} \rightleftharpoons \text{K} \rightleftharpoons \text{NH}_4$  occurring readily, whereas exchanges which aimed to introduce Ca, Ba, Cs and Li occurred at best only to a very limited extent.

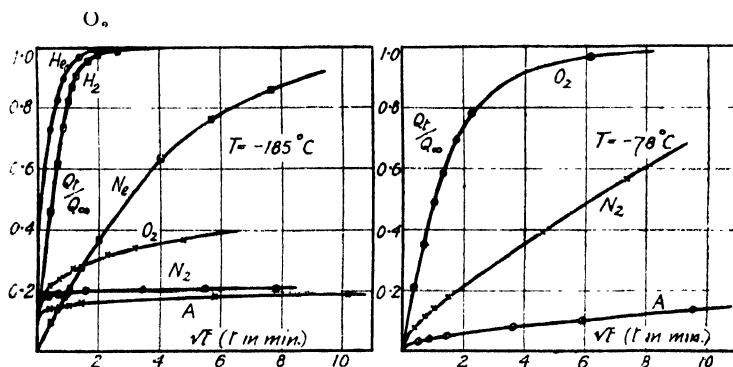


FIG. 2.—Examples of the molecular-sieve action of Ba- and Ca-mordenites towards different simple gases, as expressed by selectivity in the rates of sorption at  $-185^{\circ}\text{C}$  and  $-78^{\circ}\text{C}$ .

A study of the cation-exchanged mordenites established that, within limits set by the anionic aluminosilicate framework of the crystal, the exchange process yields a group of sorbents with a range of molecular-sieve properties. The powdered crystals gave very diverse sorption rates as Fig. 1 indicates. Although these rates are liable to be modified by a mean particle size which may have differed from one of these sorbents to another, the range in values of the constant  $D/a^2$  ( $a$  = mean radius of particle,  $D$  = diffusion coefficient) is greater than anything to be anticipated on this count. In general, in any one sorbent the rate sequence which tended to be preserved was  $\text{Kr} < \text{A} < \text{N}_2 < \text{O}_2$ ,  $\text{Ne} < \text{H}_2 < \text{He}$ , while the affinity between sorbent and sorbate was approximately in the converse order.

By cooling the sorbents to low temperatures ( $-78^{\circ}$ ,  $-186^{\circ}$ ,  $-195^{\circ}\text{C}$ ) one may often greatly alter the sorption rate of one gas relative to another,<sup>9, 14</sup> and also augment the amounts of each gas occluded at equili-

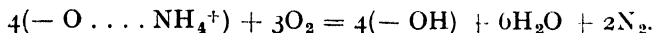
<sup>13</sup> Barrer, *J. Chem. Soc.*, 1948, 2158.

<sup>14</sup> Idem, *Nature*, 1947, **159**, 508.

\* Except in the case of  $\text{NH}_4^+$ , when vapour-phase exchange is the simplest procedure, using  $\text{NH}_4\text{Cl}$  as exchanging salt. Exchange by heating the zeolite with fused low-melting salts is also often very satisfactory.

brium. Examples of the differences in sorption rates are indicated in Fig. 2, which illustrates the magnitude of the molecular-sieve effect, even where the dimensions of the diffusing molecules differ only slightly.

A second method of altering the dimensions of the interstitial channels, and hence of modifying the molecular-sieve behaviour of zeolites, is limited to ammonium ion-exchanged crystals which are capable of occluding oxygen gas.<sup>10</sup> It has been found possible slowly to oxidize the ammonium ions with oxygen gas at  $\sim 350^\circ\text{C}$ :



Since the  $-\text{OH}$  group occupies a smaller volume than the  $-\text{O} \dots \text{NH}_4^+$  group this method, as far as it has been investigated, has led to crystals with more readily accessible or more open intracrystalline channels than occurred in the parent  $\text{NH}_4$ -zeolites. A great deal of study remains to be done before the potentialities of the ion-exchange and oxidation methods are fully evaluated in the production of special purpose sorbents for effecting molecular-sieve separations of particular mixtures. It has, however, already been shown possible to produce a great diversity of graded molecular sieves, and that quite small modifications may result in important variations in relative sorption rates, variations which may often be augmented still more by an appropriate choice of the experimental temperature. The production of modified zeolites already seems to have reached a point permitting separations of species in which only minor differences in dimensions arise ( $\text{N}_2$ - $\text{O}_2$ ;  $\text{A-O}_2$ ;  $\text{A-N}_2$ ;  $\text{Ne-H}_2$ , etc.) although actual separations have not yet been carried out.\*

**Activation and Poisoning of Zeolites.**—It has already been pointed out that the zeolites should be used as finely divided powders which are well outgassed, because the interstitial water may modify the amounts and velocities of occlusion. Several attempted separations using chabazite and involving acetone proved to be unsuccessful, perhaps because acetone which was sorbed only excessively slowly nevertheless blocked the entrance to the interstitial channels and prevented uptake of species which in absence of acetone would have been fully occluded. Little systematic work has yet been done on interference effects which may arise between individual pairs of molecules both of which are sorbed but at different velocities. In general one would expect such interference to be less important if the rapidly diffusing species were more polar than the slowly diffusing molecule because the polar species should show a higher affinity for the intracrystalline sites and so a greater interstitial concentration. The zeolites can also be poisoned when one constituent of the molecular mixture tends to decompose at the temperature of the experiment giving carbonaceous deposits on the surface of the sorbent particles. Such deposits can be burnt off using air or oxygen and the sorptive power of the zeolite restored at least in part.

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\* Work is now in progress concerned with this aspect.

# MODIFIED ACTIVATED CARBON ADSORBENTS FOR CONTINUOUS FRACTIONAL ADSORPTION

BY D. E. WEISS

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The properties of H and L carbons are briefly discussed and summarized, and a distinction drawn between the short- and long-range adsorptive forces of activated carbons. Adsorption of electrolytes on L carbons is due to electrovalent and short-range forces in alkaline solutions, and to short-range forces alone in acid solutions. The addition of some non-electrolytes increases the selectivity of the adsorbent and a theory is proposed ascribing the effect to a change in the dielectric constant in the vicinity of the carbon surface. Fatty acid films on L carbon behave as non-electrolytes in acid solutions, but form salts with cations in alkaline solutions. Cations may be salted out onto an L carbon surface at a low pH by the addition of suitable counter ions to the solution. A new technique for continuous fractional adsorption is briefly described.

It is the purpose of this paper to describe the preparation, properties and use of activated carbons modified in such a manner that they are suitable for use in chromatographic processes and in a new continuous fractional adsorption column technique at present under development by the author. The proposed mechanism of adsorption on modified adsorbents may be useful in interpreting reported anomalies encountered in partition chromatography.

## Theories of Adsorption on Activated Carbon

Michaelis and Rona<sup>1</sup> proved the adsorption of electrolytes on activated carbon to be an exchange phenomenon. In 1929 Kruyt and de Kadt<sup>2</sup> suggested, and Schilow and Tschmutow<sup>3</sup> developed the oxide theory according to which activated carbons adsorb cations and anions by salt formation with surface acidic and basic oxides; however, basic oxides of carbon of a corresponding stability are unknown in organic chemistry. Frumkin and co-worker<sup>4</sup> proposed an electrochemical theory in which an aqueous suspension of carbon in the presence of molecular oxygen acts as an oxygen electrode, becomes positively charged, and permits the adsorption of anions. Objections to this theory are that a completely out-gassed carbon activated at 1000°C can adsorb hydrochloric acid even in the absence of molecular oxygen, and that an exponential relationship between the partial pressure of the oxygen in the system and the adsorption of anions cannot be experimentally demonstrated.

Bartell and Miller,<sup>5</sup> King<sup>6</sup> and Steenberg,<sup>7</sup> have shown that activated carbons may be classified as either L or H carbons. Steenberg, who confirmed

<sup>1</sup> Michaelis and Rona, *Biochem. Z.*, 1920, **102**, 268.

<sup>2</sup> Kruyt and de Kadt, *Kolloid-Beih.*, 1931, **32**, 249.

<sup>3</sup> Schilow and Tschmutow, *Z. physik. Chem. A*, 1930, **148**, 233.

<sup>4</sup> Bruns and Frumkin, *Z. physik. Chem. A*, 1929, **141**, 141.

<sup>5</sup> Bartell and Miller, *J. Physic. Chem.*, 1924, **28**, 992.

<sup>6</sup> King, *J. Chem. Soc.*, 1937, 1489.

<sup>7</sup> Steenberg, *Adsorption and Exchange of Ions and Activated Charcoal* (Alinquist and Wiksells, Uppsala, 1944).

their results for H carbons, defined an L carbon as one that could, and an H carbon as one that could not adsorb sodium hydroxide from an aqueous solution.

**The Properties of H Carbons.**—Steenberg showed that anions were adsorbed by van der Waals' forces on H carbons whereas cations were adsorbed electrovalently by L carbons and distinguished between ions primarily and secondarily adsorbed on H carbons. Primarily adsorbed ions are those held to the carbon surface by the short-range adsorptive forces and which attract a diffuse ionic atmosphere of secondarily adsorbed counter ions. The secondarily adsorbed inorganic ions, but not the primarily adsorbed ions, can be exchanged for other inorganic ions depending on their position in the lyotropic series and the concentration of the exchanging ions. Application of the Boltzmann distribution law to the adsorption of

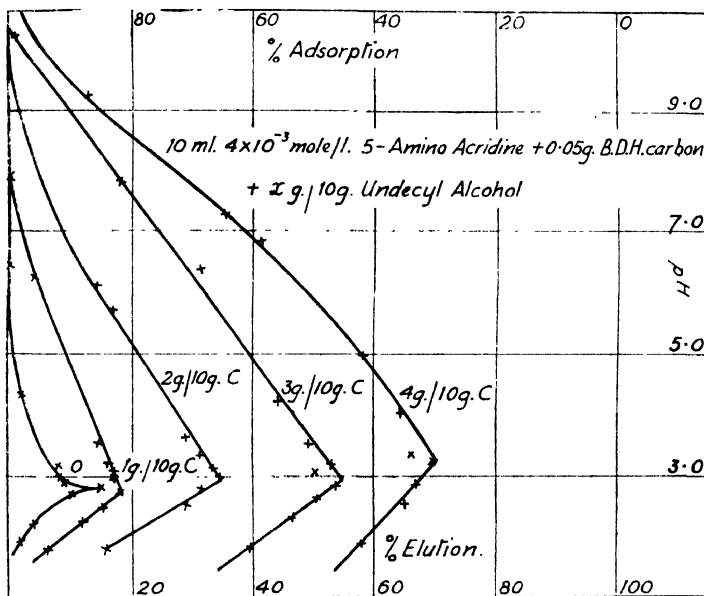


FIG. 1.

ions on H carbons yields a relationship between the primary adsorption of an ion and the concentration and valency of its counter ions. Similar ideas were independently advanced by Weiss,<sup>8</sup> who was unaware of the work of Steenberg, and who showed that an activated carbon containing primarily adsorbed oleic acid functioned as an ion-exchange adsorbent for 5-amino-acridine cations.

Steenberg has also shown that all inorganic anions (with the exception of the hydroxyl ion), but no inorganic cations (with the exception of the hydrogen ion) can be primarily adsorbed; and thus the adsorption of hydrochloric acid by H carbons is due to the primary adsorption of hydrogen ions, and the secondary adsorption of chloride ions. Similarly the weak primary adsorption of chloride ions accounts for the weak adsorption of sodium chloride by an H carbon although it cannot adsorb sodium hydroxide. Primary adsorption of inorganic ions is reduced by the adsorption of organic ions, irrespective of their electrical charge.

<sup>8</sup> Weiss, *Nature*, 1948, **162**, 372.

The pH variations <sup>5</sup> associated with adsorption of salts on H carbons can be interpreted in terms of the Donnan equilibrium. When the activity of the secondarily adsorbed or counter ions is comparable with that of the hydrogen or hydroxyl ions of water, competitive secondary adsorption with the counter ions occurs, with a resultant alteration in the pH of the original aqueous suspension. If the salt concentration of the suspension is increased this competitive effect diminishes.

**The Properties of L Carbons.**—L carbons may be prepared at lower activation temperatures than H carbons as shown by King <sup>6</sup> and Steenberg. <sup>7</sup> Low activation temperatures produced L carbons, the alkali-adsorbing capacity of which increased with decrease in activation temperature and which was shown by Weller and Young <sup>9</sup> to vary inversely with its ability to adsorb acid. The pH of aqueous suspensions of L carbons so prepared

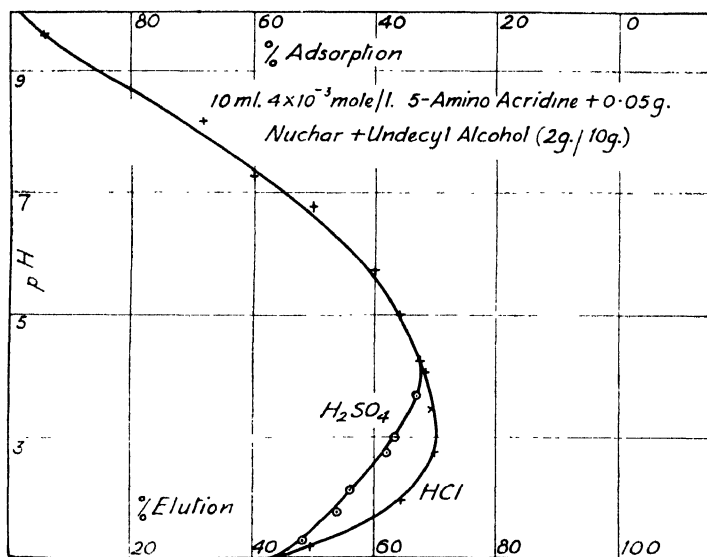


FIG. 2.

decreased with decrease in activation temperature and suggests that the degree of surface oxidation increases with decrease in activation temperature, an assumption supported by the analytical data of Weller and Young.

Hauge and Willaman <sup>10</sup> and Sastri <sup>11</sup> showed that typical activated carbons, presumably L carbons, exhibited maximum adsorption of electro-negative substances from aqueous solutions at a low pH, and of electro-positive substances at a high pH. The adsorption of these electrolytes was a minimum in the region of a pH zone termed the isoelectric zone but the adsorption of electrically neutral substances was unaffected by pH. Olin, Lykins and Munro <sup>12</sup> deduced from cataphoretic experiments that L carbons are electrokinetically negative at high pH values but electropositive at low pH values.

<sup>9</sup> Weller and Young, *J. Amer. Chem. Soc.*, 1948, **70**, 4155.

<sup>10</sup> Hauge and Willaman, *Ind. Eng. Chem.*, 1927, **19**, 943.

<sup>11</sup> Sastri, *Quart. J. Indian Sci.*, 1942, **5**, 107.

<sup>12</sup> Olin, Lykins and Munro, *Ind. Eng. Chem.*, 1935, **27**, 690.

**The Adsorptive Forces on Activated Carbons.**—It is important to distinguish between the short- and long-range forces of adsorption operating at a carbon surface. The short range or van der Waals' forces only extend a distance of about 5 Å from the surface and are highly specific, being greatly influenced by the molecular configuration of the adsorbate and adsorbent. The long-range or electrovalent forces of adsorption, which arise from ionization of a chemically bound radical on the carbon surface or from primarily adsorbed ions, extend to distances up to 100 Å from the carbon surface and are relatively non-specific.

### Modified Activated Carbon Adsorbents

The properties of activated L carbons may be utilized to prepare versatile modified adsorbents. A film of non-electrolyte or fatty acid, defined as the modifying agent, is adsorbed on the carbon. The adsorption and desorption

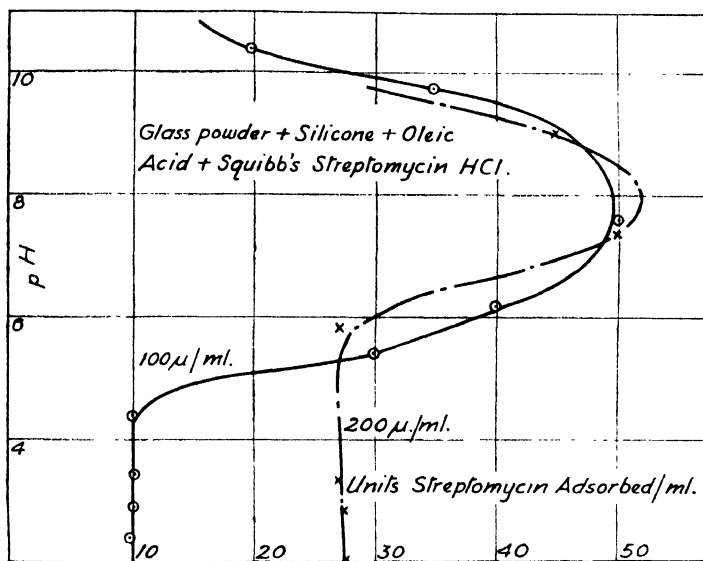


FIG. 3.

of electrolytes from aqueous solutions may then be closely controlled by pH adjustments and the possibilities of preferential adsorption are enhanced. The adsorption of 5-aminoacridine, a conjugated basic molecule, and streptomycin, a non-conjugated basic molecule, has been studied on these modified adsorbents. The addition of undecyl alcohol to an L carbon enables strong electrovalent adsorption of 5-aminoacridine to occur at pH 9, but the adsorption decreases with decrease in pH to a minimum value below which adsorption increases with further decrease in pH. Adsorption in this low pH region is probably due to the short-range forces, a view supported by comparison of the adsorption of 5-aminoacridine and streptomycin on modified adsorbents in this pH region. Adsorption of streptomycin on films of oleic acid supported on hydrophobic glass or H carbon has shown that the acid film behaves as a cation exchange adsorbent over a limited range of pH, but that at low pH values the oleic acid film behaves in an essentially similar manner to the carbon modified with undecyl alcohol. Similar behaviour occurs when the oleic acid is supported on an L carbon but is complicated by the electrovalent forces arising from dissociation, under alkaline conditions, of the surface acidic oxides of the carbon.

**Addition to Non-electrolytes to L Carbons.**—Fig. 1\* shows the relation between pH and the adsorption of 5-aminoacridine hydrochloride on an L B.D.H. carbon and on this carbon modified with undecyl alcohol. The curves show that modification with undecyl alcohol reduces the adsorption of the organic cation, particularly at pH 2.8, the pH of minimum adsorption. At this pH the surface oxides of the carbon are only slightly ionized, and the carbon behaves as an H carbon.

The effect of the alcohol is considered to be threefold; it competes with the cations for adsorption by the short-range forces, it acts as a solvent for the cation, and it reduces the dielectric constant in the immediate vicinity of the carbon surface. Since the alcohol may form a hydrogen bond with the heterocyclic nitrogen atoms of the 5-aminoacridine to produce an alcohol-acridine complex at the carbon surface, carbons modified by the addition

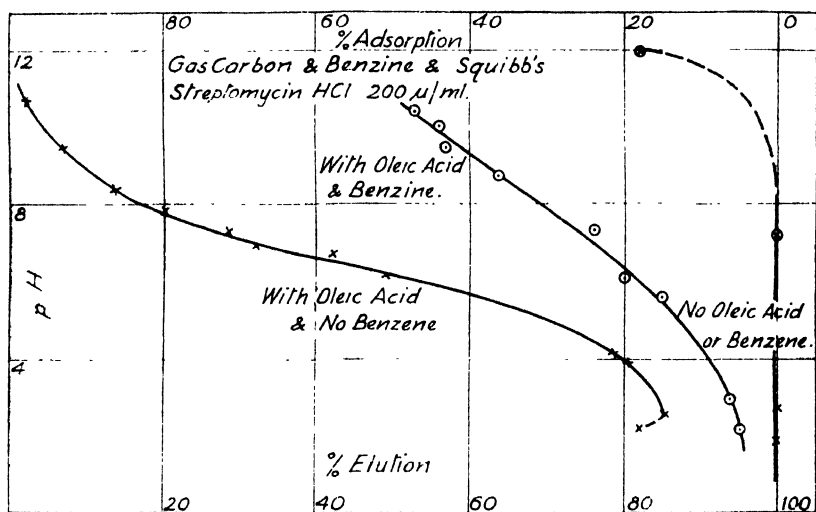


FIG. 4.

of small amounts of undecyl alcohol may be pictured as consisting of primarily adsorbed acridine ions surrounded by alcohol molecules which prevent the further approach of hydrated acridine ions from the solution. Equilibrium is reached when the tendency for the primary adsorption of the 5-aminoacridine ions is counterbalanced by the electrostatic repulsive forces of the interfacial potential produced by adsorption, and the electrically charged carbon surface may be regarded as one plate of a condenser. Addition of undecyl alcohol diminishes the dielectric constant in the vicinity of the carbon surface, so reducing the primary adsorption of ions necessary to maintain the equilibrium electrostatic repulsive force. Increasing the alcohol film thickness reduces the effect of lowering the dielectric constant in the vicinity of the carbon surface since a greater proportion of the approaching ions will be surrounded by a liquid of the same dielectric constant.

In addition to their effect on the dielectric constant in the vicinity of the carbon surface, the alcohol molecules probably competitively displace some of the adsorbed 5-aminoacridine ions from the carbon surface. If the

\* The curves were obtained by equilibrating 0.05 g. samples of the modified adsorbent with 10 ml. aliquots of the solution, centrifuging, and estimating the 5-aminoacridine in the supernatant liquid at 390 m $\mu$  in a Beckman spectrophotometer.

effect of the undecyl alcohol were only to produce a film in which the cations were soluble, so that adsorption of the cations were simply a partition between two solvents, the adsorption of 5-aminoacridine should increase with increase in alcohol concentration whereas the experimental results show that the reverse actually occurs. When the adsorbed ions are completely immiscible with the modifying adsorbate film, zero adsorption of the ions will occur if the modifying adsorbate is more strongly adsorbed.

These concepts are supported by some observations of Steenberg <sup>7</sup> who showed that Nile Blue adsorbed on an H carbon was desorbed on shaking with a benzene-water emulsion since Nile Blue is insoluble in benzene. When atabrin was primarily adsorbed instead of Nile Blue, no desorption occurred after shaking with benzene and water, since atabrin is soluble in benzene.

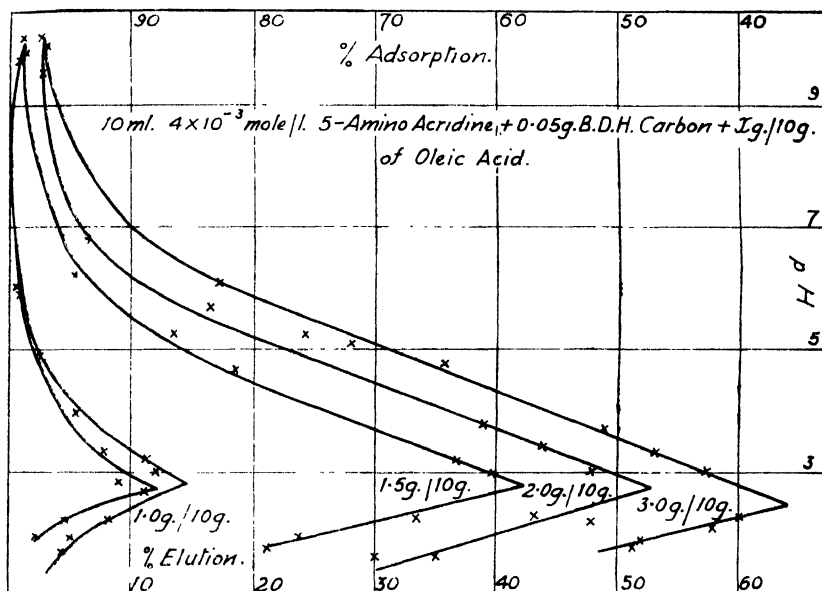


FIG. 5.

The increase in adsorption of 5-aminoacridine as the pH is raised from the minimum at 2.8 results from ionization of the acidic surface oxides, which increases the electrostatic negative charge, so attracting additional 5-aminoacridine ions to the carbon surface where some are adsorbed primarily by the short-range forces, and others are adsorbed electrovalently to form an ionic atmosphere in the vicinity of the ionized oxide groups. Hence the adsorption of 5-aminoacridine will increase with increase in pH to a maximum, where complete ionization of the surface oxides occurs. The primarily adsorbed ions will in turn attract an ionic atmosphere of chloride or hydroxyl ions.

The observations of Steenberg with H carbons show that the primary adsorption of an ion can be increased by increasing the valency or concentration of its counter ions. Increasing the chloride ion concentration by the addition of hydrochloric acid increases the primary adsorption of the 5-aminoacridine hydrochloride below pH 2.8: here the carbon behaves essentially as an H carbon. Fig. 2 compares the adsorption of 5-aminoacridine on the L carbon (Nuchar), modified with undecyl alcohol, when the pH is adjusted with hydrochloric or with sulphuric acid, and shows that the same



amount of primary adsorption of 5-aminoacridine occurs with a lower concentration of sulphate than chloride ions. Hence the pH for minimum adsorption of a cation on an L carbon depends on the degree of surface oxidation of the carbon, and on the nature of the anions present in the solution.

These findings are in agreement with the experimental results of previous investigations on the adsorption of electrolytes on activated carbons: the addition of suitable modifying adsorbates merely increases the selectivity of the adsorption. Further, these conclusions account for the observations of Olin, Lykins and Munro,<sup>12</sup> mentioned above, on the electrokinetic properties of L carbons, which are governed by the net electrical charge at the carbon surface. Increase in the valency or the concentration of the counter ions will decrease the positive electrokinetic potential by reducing the extent of the diffuse double layer.

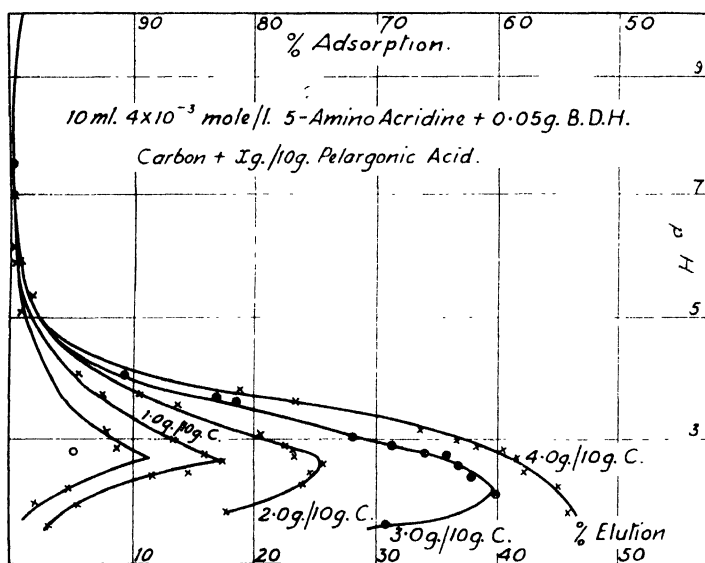


FIG. 6.

**The Adsorption of Cations on Fatty Acid Films on Glass and H Carbons.**—Fig. 3 shows the relation between pH and the adsorption of Squibb's streptomycin hydrochloride\* on a hydrophobic glass powder† modified with oleic acid and shows that oleic acid adsorbed at a hydrophobic surface can act as a cation exchange adsorbent for streptomycin over the pH range in which oleic acid is ionized. The weak adsorption of streptomycin below pH 4 may be due to hydrogen bonding between the hydroxyl groups of the streptomycin and the undissociated carboxylic groups of the acid.

Fig. 4 shows the relation between pH and the adsorption of streptomycin on a steam-activated H carbon modified with oleic acid. Unlike the hydro-

<sup>12</sup> Titus and Fried, *J. Biol. Chem.*, 1948, **174**, 57.

\* The streptomycin solutions were assayed by the maltol method of Titus and Fried.<sup>12</sup>

† In the experiments discussed in this section hydrophobic glass powder was prepared by treating glass powder with a benzene solution of Drifilm, a silicone preparation.

phobic glass powder modified with oleic acid, adsorption is almost complete in the higher pH range although streptomycin should be present as the free base in this pH region. Exposure of the H carbon to benzene vapour before modification with oleic acid resulted in a relatively high adsorption of streptomycin at pH 8-10 although it might be expected that the surface would behave in a similar manner to that of the hydrophobic glass modified with oleic acid.

#### The Adsorption of Cations on Fatty Acid Films on L Carbons.—

Fig. 5 and 6 show the relation between pH and the adsorption of 5-aminoacridine hydrochloride on B.D.H. L carbon modified with a fatty acid. At pH 2.8 the unionized fatty acid functions similarly to undecyl alcohol and the increased adsorption of 5-aminoacridine with decrease in pH is due to the effect of the anions added during pH adjustment. The effect of increasing the pH on the adsorption of 5-aminoacridine is twofold: as the

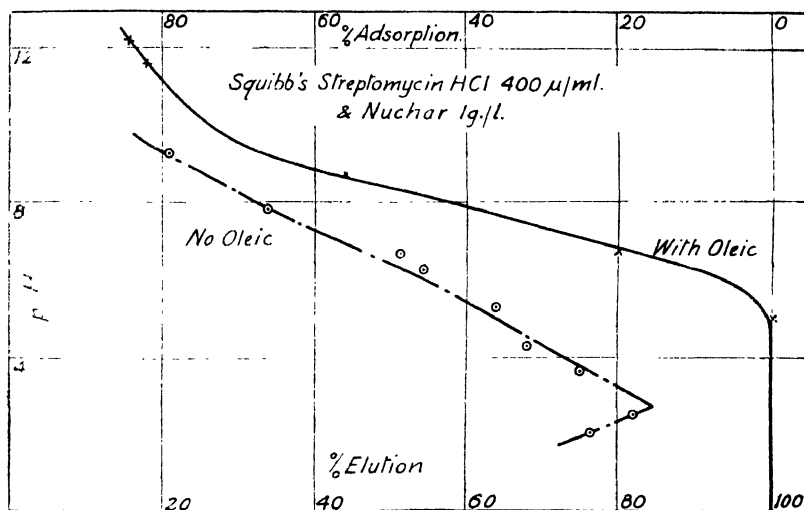


FIG. 7.

pH is increased above 2.8, 5-aminoacridine is adsorbed electrovalently both by the oleic acid and the surface oxides. Above pH 9-10 the ionization of the 5-aminoacridine is reduced, and the free base is precipitated onto the carbon surface. Fig. 7 and 8 show that the adsorption of the streptomycin cation on Nuchar and B.D.H. carbon treated with oleic acid does not increase with decrease in pH, possibly since the streptomycin cation with its carbohydrate structure has no affinity for, and cannot be primarily adsorbed in the hydrophobic oleic acid film.

#### Discussion

The foregoing results demonstrate that the adsorptive properties of activated carbons may be modified in a great many ways by the addition of a suitable modifying adsorbate. An H carbon can be converted to a cation or anion exchange adsorbent with an exchange capacity comparable with that of the synthetic ion exchange resins. Many substances other than oleic acid may be used as modifying adsorbates. Thus the modification of an H carbon with picric acid, methyl dioctylamine, or a quaternary ammonium dyestuff produces an exchange adsorbent capable of removing inorganic

cations, acids, or anions from aqueous solutions. Application to the adsorption of organic ions is more limited since a modifying adsorbate must be chosen which is not desorbed by the organic ions to be exchanged. Exchange of organic ions is greatly influenced by the short-range forces of adsorption, and hence by the molecular structure of the modifying adsorbate. A judicious choice of modifying adsorbates permits the preparation of ion exchange adsorbents of great versatility and selectivity, and which, unlike the synthetic ion exchange resins, do not swell upon change of pH, a feature of considerable practical interest.

The difficulty of desorbing a material after adsorption on activated carbons may be overcome by either of two general methods. The carbon containing the adsorbate can be shaken with an emulsion in water of an organic liquid in which the adsorbate is insoluble, so expelling the adsorbate into the aqueous solution. This technique, which can be used for non-electrolytes as well as for electrolytes, enables highly concentrated eluates to be produced

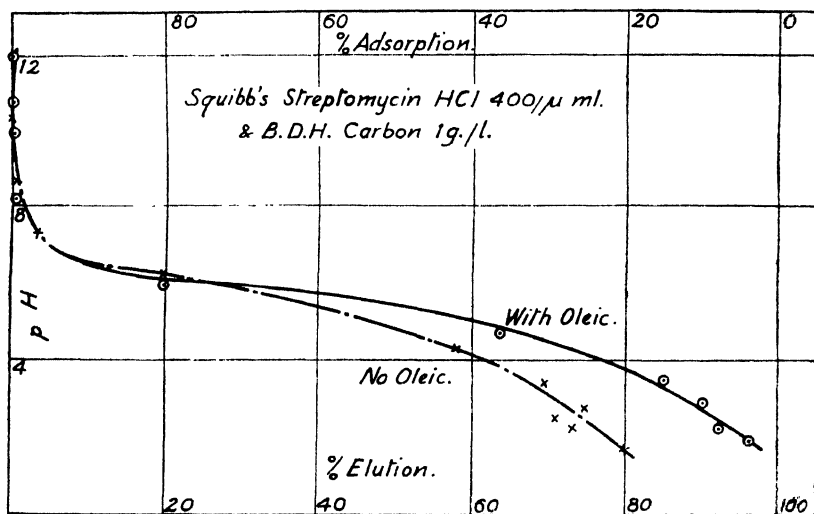


FIG. 8.

with the use of a very small volume of organic solvent, but has the disadvantage that the carbon is not simultaneously regenerated. The alternative procedure is to modify an L carbon with a suitable adsorbate film with which the materials to be adsorbed are partially miscible. Adsorption and desorption of electrolytes on such an adsorbent can then be controlled by adjusting the pH or ionic strength of the solution. Adsorption at a high pH takes advantage of both the short- and long-range adsorptive forces of an L carbon surface, and can be used to separate cations from anions and non-electrolytes. Adsorption at a low pH in the presence of a suitable modifying adsorbate utilizes the more selective short-range forces. For example, 5-aminoacridine can be adsorbed in the presence of oleic acid at pH 2, whereas streptomycin is completely desorbed at this pH, although both are strongly adsorbed at pH 9.

These adsorbents possess two important properties; desorption by pH adjustment results in simple regeneration of the adsorbent and a suitability for use in a continuous fractional adsorption technique at present being developed in this laboratory. For use in this technique, modifying adsorbates must be chosen which not only confer the desired adsorptive properties

on the carbon surface but also condition it for froth flotation. The "floating" adsorbent may be contacted continuously in a column with a descending liquid stream containing the substances to be fractionated. The feed solution is introduced midway in the fractionating column, and divides it into rectifying and stripping sections. Rectification is obtained by maintaining a pH gradient over the rectifying section, in which a partial stripping of the adsorbate occurs as the adsorbent ascends. The adsorbate retained by the adsorbent at the top of the rectifying section is desorbed by a further pH adjustment, enabling the whole operation to be conducted in an aqueous solution and the adsorbent to be re-cycled.

The author gratefully acknowledges the able assistance of Miss H. Doery\* with the experimental streptomycin work and A. L. Odgers with the remaining experimental work. The work described in this paper is part of the research programme of the Chemical Engineering Section of the Division of Industrial Chemistry, C.S.I.R.O., Australia.

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### GENERAL DISCUSSION \*

**Dr. A. J. P. Martin** (*Medical Research Council Unit, London*) said: The easy separation of *cis* and *trans* isomers of carotenoids on adsorption columns contrasts with the almost complete identity of behaviour of oleic and elaidic acids on partition columns. Is information available as to partition coefficients of the carotenoid isomers? Since all the isomers would seem to allow easy approach of solvent molecules from all sides, little difference in partition coefficient would be expected.

**Prof. L. Zechmeister** (*California*) said: Dr. Martin has asked the question whether classical chromatography or partition chromatography is more efficient in the separation of *cis-trans* isomers. This question cannot be answered at the present time, since, because of the high efficiency of ordinary chromatography, the partition method has not yet been tested. However, it seems to be useful to make the following comments.

We may differentiate between (a) morphologically sensitive stereo-isomeric sets (for example, a single *trans*→*cis* rearrangement may thoroughly alter the molecular shape of a polyene), and (b) morphologically less sensitive sets (for example, the overall shape of a sugar molecule is not essentially altered by epimerization). It is suggested that the chances for successful resolutions of stereo-isomeric mixtures by classical chromatography are greater for type (a) than for (b).

**Prof. A. Tiselius** (*Uppsala*) said: The results reported by Prof. Zechmeister and Prof. Brockmann appear to me to be particularly valuable as an illustration of what factors are of importance for the selectivity of adsorption chromatographic methods. In this connection I would like to raise the question which of the two phenomena, adsorption or partition (and one ought also to include ionic exchange), is the most specific—a question which should be of great practical importance in the planning of work in this field.

**Dr. A. Klinkenberg** (*The Hague*) said: In the paper by Stewart data have been given on the band-widening caused by the passage of a fluid through

\* On remaining papers of Section I.

a packed column. I should like to draw attention to the following way of approach to a description of this phenomenon, worked out for the case of a linear isotherm.<sup>1</sup>

The authors of theoretical papers on chromatographic adsorption and other percolation processes, such as transient heat transfer in a packed bed, ignore in their calculations the disturbing effect of non-uniformity of forward velocity and then develop a differential equation involving a constant value of this velocity. Of course the velocity in reality varies from wall to axis in any capillary and it will also differ for pores of different radii.

I separated this effect from the other cause for the levelling-off of concentration gradients, viz., the finite rate of mass transfer (non-attainment of equilibrium), by studying cases, where mass transfer was definitely ruled out. To this end solvent was displaced by solution, or vice versa, in a packing of non-porous particles of 80-100 mesh. The effect was quite considerable. The plot of the effluent concentration against volume collected resembled the S-shaped curve of the probability integral.

This can be understood with the aid of the following model. The packing is assumed to consist of layers of particles, the passage through each of which producing a certain distribution of times of passage. Complete mixing occurs between layers, so that successive distributions are uncorrelated. The distribution of times of passage for the aggregate of many layers will then approach a Gaussian probability curve, i.e., an instantaneous introduction of solute will give rise to a Gauss curve in the effluent and a concentration jump to the probability integral. The same of course applies to distributions in terms of volumes of effluent.

Another approach is obtained by introducing an apparent diffusivity

$$D_{app.} = cD_p v,$$

where  $D_p$  = particle diameter,  
 $v$  = mean linear velocity calculated on free space,  
 $c$  = dimensionless constant, characterizing the packing,

as has been made by Schmidt and Damköhler<sup>2</sup> for radial conduction of heat and Bernard<sup>3</sup> for radial transport of solute.

This again leads to Gaussian distributions, provided the column is long enough. But for the interpretation of the dimensionless constant the equations are identical. The value of  $v$  is found to have no effect on the distribution, and the standard deviation of the distribution, expressed in volumes of solution, is proportional to the square root of height  $H$  over particle size  $D_p$ .

Some very fine examples of Gauss curves are indeed to be found in literature, e.g.,

(i) Weil-Malherbe,<sup>4</sup> benzpyrene in light petroleum containing 3 % of benzene, percolated through silica.

(ii) Moore and Stein,<sup>5</sup> partition chromatography of amino acids on starch.

The values of the constant  $c$  calculated from these curves were of the same order as, and certainly not much higher than, the  $c$  values obtained from displacement experiments in non-porous beds, packed with good care. It was therefore concluded that the disturbing effect, caused by the variations in forward velocity may be an important contribution to the total band-widening.

It is recalled that the stage concept (Martin and Synge<sup>6</sup>) and the concept of finite rate of mass transfer (Schumann-Furnas theory) for long columns also lead to Gauss distributions, so that it is impossible to decide on a mechanism on the strength of a single observed curve. The number of stages calculated according to Martin and Synge is closely related to the number of layers of particles,  $H/D_p$ ; in fact, it is found to equal  $H/2cD_p$ .

Trends other than those given by Levi have been developed in Holland since 1943, when an independent discovery of partition chromatography was

<sup>1</sup> To be published in *Trans. Faraday Soc.*

<sup>2</sup> Damköhler, *Z. Elektrochem.*, 1936, **42**, 859.

<sup>3</sup> Bernard, *Thesis* (Princeton Univ., 1948).

<sup>4</sup> Weil-Malherbe, *J. Chem. Soc.*, 1943, 303.

<sup>5</sup> Moore and Stein, *Ann. N.Y. Acad. Sci.*, 1948, **49**, 161.

<sup>6</sup> Martin and Synge, *Biochem. J.*, 1941, **35**, 1358.

made by Van Dijck and a patent application filed. Subsequently through references, Martin and Synge's work became known.

We tried to do away with adsorption as a disturbing factor more radically than did Martin and co-workers, and used non-porous solids with adhering liquid, e.g., in a typical experiment a packing consisting of 222 ml. of sand (70-120 mesh), 27.5 ml. water and 104 ml. of an ether-pentane 50/50 mixture. With this packing hydroquinone and pyrocatechol were separated successfully. The packing was obtained from a water-filled sand by displacement by the organic liquid.

In order to have the polar liquid moving, the oleophilic solid carborundum was used, which is commercially available in carefully screened fractions. Benzene in this case was the stationary fluid. With suitable buffer mixtures as the moving fluid the system was used for some separations of alkaloids.

**Mr. C. D. Cook** (*Widnes*) said: I would like to confirm certain observations made by Mr. Stewart on the subject of band widening as the solute travels down the column. As Mr. Stewart has pointed out, theory has it that the volume of the band should not increase during elution, but, using compounds with linear isotherms and the partition systems, (a) *n*-hexane-nitromethane-silica, and (b) chloroform-water-silica, I have found that the rate of increase of the volume of the band in the percolate was proportional to the volume required to elute the maximum concentration of the solute. Although the volume of the band was dependent on the weight of the solute in the sample, it was independent of the partition coefficient.

It was found possible to derive a simple, linear expression, based on experimental work, to calculate the volume of the band in the percolate when (a) the volume required to elute the maximum concentration of the solute, (b) the weight of the solute present, and (c) certain characteristics depending on the silica and solute are known.

The volume of the band was found to increase slightly when the rate of flow of the eluent was caused to decrease from 5 ml./min. to 50 ml./hr. A rapid increase in the band width was observed when the rate of flow was further diminished from 10 ml./hr. to 1 ml./hr. This rapid band widening was, I believe, partially due to vertical diffusion. The volume of the band, likewise, increased with increasing volume of the solution of the sample when loaded on to the column.

By varying the weights of silica, nitromethane and using components of differing partition coefficients, the following expression was derived:

$$V_E = V_g + N(\alpha - 1) - \frac{w}{\rho}, \quad (1)$$

where  $V_E$  = the volume required to elute the maximum concentration of the solute,

$V_g$  = the gel volume,

$w$  = wt. of silica,

$\rho$  = density of silica,

$N$  = volume of immobile phase,

$\alpha$  = the partition coefficient.

This expression, which has been found to be obeyed with considerable accuracy, is identical to the Martin and Synge expression:<sup>7</sup>

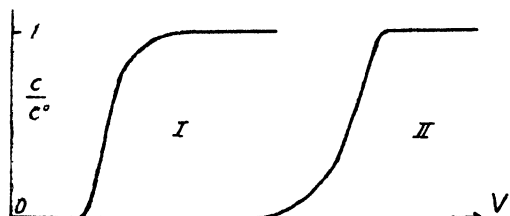
$$\alpha = \frac{A}{RA_s} - \frac{A_L}{A_s},$$

but I believe it is in a more useful form.

Since the volume of the band is dependent on  $V_E$  (eqn. (1)), it is essential that a silica of low gel volume be used to effect a maximum resolution of two components whose partition coefficients do not differ appreciably. We, therefore, consider that the gel volume of silicas of similar sieve range is one of the more important characteristics of a silica used in partition chromatography. I hope to have more to say on the characterization of silicas used in partition chromatography at a later date.

<sup>7</sup> Martin and Synge, *Biochem. J.*, 1941, **35**, 1358.

**Dr. E. Glueckauf** (*A.E.R.E., Harwell*) (*communicated*): It usually requires a fair amount of kinetic experimental data to decide, in the case of non-equilibrium, whether grain-diffusion (I) or diffusion through a liquid film (II) is the rate-determining factor. It may therefore be of interest to know that in the

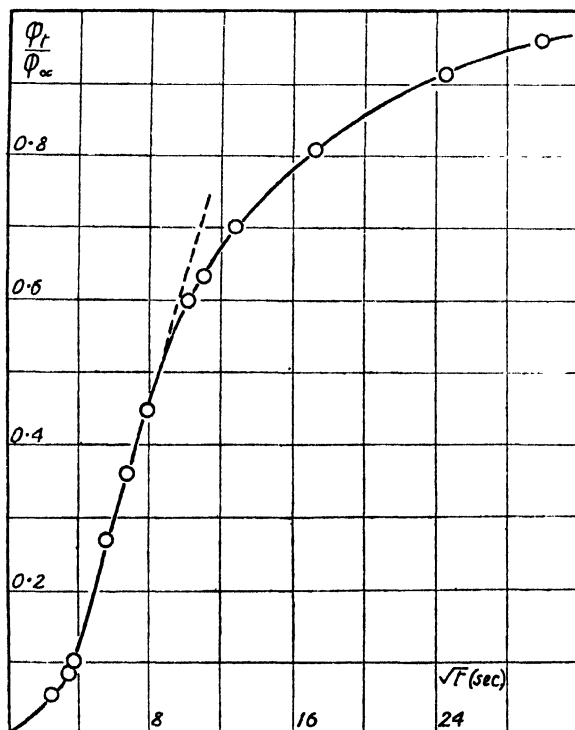


Elution boundary in the case of non-equilibrium due to I grain-diffusion, II liquid-film diffusion.

case of isotherms concave against the  $c$ -axis the form of a self-sharpening front boundary obtained during frontal analysis has a different form, according to which of these processes is predominant. It follows from eqn. (3), (3c) and (4) of our paper<sup>\*</sup> that in these two cases this boundary has the forms I and II (see Fig.) respectively, which should make recognition easy. We were

able to confirm this by experiments with ion-exchangers using greatly different concentrations. Using all possible starting points  $C_1$ – $C_2$  we get thus two families of curves in a  $C_1$ – $C_2$  diagram. Examples in the case of two isotherms are shown<sup>\*</sup> in Fig. 2 and 3.

**Mr. B. A. J. Lister and Dr. J. F. Duncan** (*A.E.R.E., Harwell*) said: The results of Mr. Kressman and Dr. Kitchener suggest that under a given set of conditions one mechanism of diffusion only is operative and that film-diffusion



Particle diffusion equation curve for the uptake of cerous ion on Zeo-Karb 215 from 0.1 N  $\text{Ce}(\text{NO}_3)_3$  solution.

<sup>\*</sup> Glueckauf, Barker and Kitt, This Discussion.

and particle-diffusion kinetics are sufficiently different to be readily distinguished by conformity to one of two equations.

An examination of the graphs in their paper shows that in almost every case illustrating particle diffusion (Fig. 7 (a), 8 and 9) the experimental points at lower values of  $Q_t/Q_\infty$  do not fall on straight lines passing directly through the origin but deviate from linearity in a regular manner. Curve 7 in Fig. 6 is described as film mechanism although it does not appear to differ essentially in shape from curves drawn through the experimental points illustrating particle diffusion in Fig. 7, 8 and 9. A completely different mechanism does not appear to be necessary especially since experimental points were determined only over the range  $0.2 < Q_t/Q_\infty < 0.9$ .

An examination of some results (B. A. J. L.) on the rate of uptake of cerous ion on Zeo-Karb 215 (among other exchangers) shows a similarly shaped curve (see Fig.). Here a linear portion in the particle diffusion curve was obtained but this was not directed immediately towards the origin. The slower initial uptake has also been found by Kressman and Kitchener although not emphasized in their graphs. It may be due to a variety of causes such as swelling, variation in the diffusion constant, slow saturation of the surface with cations or simultaneous occurrence of film and particle diffusion. Further experimental work is necessary to distinguish between these. It appears more difficult, therefore, than is suggested to differentiate between the two mechanisms and it seems to us that they may overlap more generally than has been supposed.

**Dr. J. A. Kitchener** (*London*) said: In reply to Mr. Lister's comments on our paper, we cannot agree that there is any real difficulty in practice in differentiating unambiguously between the particle- and film-diffusion mechanisms in a given case. (The only possible exception to this statement is in those rare circumstances where the transitional region between the two mechanisms happens to be met. Even then, the *I*-mechanism is clearly indicated since neither *P*- nor *F*-kinetics fit.) In every case so far investigated the form of the kinetics—which is used as the first test of mechanism—has proved to have given the correct indication of mechanism when interruption tests have been applied.

For example, consider the case questioned by Mr. Lister, namely, the  $\text{NH}_4^+ - \text{Na}^+$  exchange at  $25^\circ\text{C}$ . If it is not immediately apparent that curve 7 in Fig. 6 (b) is of distinctly different type from those normally obtained for particle diffusion (which should be clear since curve 7 cuts curve 8), at least when the alternative graph is plotted for the same data (see line 4, Fig. 7 (b)) the excellent straight line now obtained *over the whole range of the exchange* gives conclusive indication that the data conform to *F*-kinetics.

If further proof should be required, examine the results of interruption tests on the same system. These are included as crosses in curve 2, Fig. 5. Interruption is seen to be entirely without effect. Consequently, the process is unquestionably controlled by film diffusion.

There are thus always *three* criteria, not one, namely, (a) the results must conform to one set of kinetics, (b) they must deviate from the other, and (c) they must behave appropriately in interruption tests. In some cases, too, as we have shown, there is also the added confirmation—though never really needed to prove the mechanism—of the influence of stirring. All these criteria must lead to mutually consistent conclusions, which can therefore be accepted with complete confidence.

With regard to the fact that exchanges of the *P*-type have always tended to give a slow beginning (as can be seen in all our  $\sqrt{t}$ -graphs in Fig. 7 (c), 8 and 9 and by the departure of the results from Paterson's theoretical equation, mentioned in the paper), there is now no doubt that the effect is due to slow swelling of the initially air-dried resin grains when they are first immersed in the solution. Measurements of the mean grain cross-sectional area have been made by a photo-electric method, and have shown a slight swelling which is practically complete in about two minutes. This value is qualitatively consistent with the amount of "lag" revealed by the  $\sqrt{t}$ -graphs.

It is interesting that this extraneous complication of swelling provides unexpected additional evidence in support of the two clear-cut mechanisms. This comes about as follows. All those results which indicate *P*-mechanism



show the above-mentioned slow beginning with gradual acceleration as the particle swells. In contrast, all those exchanges which conform to *F*-kinetics (Fig. 6 (a) and 7 (b)) show no sign whatever of acceleration: even the earliest points lie well on a straight line passing through the origin. This is exactly what is to be expected, since changes of rate of diffusion *inside* the particles should have no effect on the kinetics when the rate-controlling process is diffusion through a bounding Nernst film. The influence of swelling therefore provides a *fifth* line of evidence in determining the mechanism of exchange.

**Dr. J. F. Duncan and Mr. B. A. J. Lister** (*A.E.R.E., Harwell*) said: Since our paper was written further information has become available concerning the results of other workers who have determined mass products in ion exchange systems. These are summarized:

(a) Davidson, Argersinger, Stoenner and Lowen<sup>9</sup> have obtained a curve of similar shape to Fig. 6 for the sodium-hydrogen exchange system, the maximum occurring at  $X_{\text{NaR}} = 0.23$ , and the slopes on each side of the maximum being somewhat greater.

(b) Strickland<sup>10</sup> has also obtained a maximum for the sodium-hydrogen system using Dowex-50, and a coarse Zeo-Karb, but not with a finely divided Zeo-Karb.

(c) Reichenberg<sup>11</sup> has obtained a falling value of  $K_a$  for large values of  $X_{\text{NaR}}$  (using a sulphonated polystyrene), but did not obtain a maximum.

(d) Marinsky and Coryell<sup>12</sup> observed values of  $K_a$  which rose from  $X_{\text{NaR}} = 0$  to about 0.1 (using 40–100 mesh Dowex-50) in agreement with our observations (using 100–250 Dowex-50) but no decrease in  $K_a$  was observed at higher values. The cerium-hydrogen mass-product plots obtained by these workers (using a molar fraction concept) were similar in form to that obtained in the lanthanum-ammonium system (Fig. 5), constant values of  $K_a$  being obtained over the range  $10^{-3} < X_{\text{CeR}} < 10^{-5}$ . Similar curves were also obtained for the barium- and calcium-hydrogen systems. The barium system was quite different from the curve of Fig. 4.

For the copper-hydrogen system, we have found that the value of the mass product (using both eqn. (3) and (10)) slowly approaches a constant value as  $x$  (or  $X$ ) becomes zero. If the barium-hydrogen system were studied at a low enough concentration presumably the  $K_a$  value would behave similarly.

In view of these discrepancies, it is reasonable to suppose that the conditions, or materials used by various workers differ in some unknown manner. In particular, behaviour at low values of the molar fraction of the metal ion seems anomalous, and in this respect the results of Strickland are significant. That changes in the volume of the exchanger are a contributory factor we do not doubt, since swelling of the exchanger has been shown to take place in the zinc-uranyl exchange system (see below). We do not, however, consider that this is the whole explanation of the anomalies reported above.

Investigations of the effect of swelling have been made on the zinc-uranyl exchange system, for which values of

$$K_c = \frac{X_{\text{ZnR}}}{X_{\text{ZnS}}^{++}} \times \frac{X_{\text{UO}_2^{+1}}}{X_{\text{UO}_2\text{R}}}$$

(where the symbols are the same as described above) have also been determined by gravimetrically estimating the loss of uranium from a mixture of zinc and uranyl nitrates of known total concentration when equilibrated with an exchanger of known capacity. The mass product in solutions of 0.2 N was found to vary from 0.6 to 1.90 ( $0.10 < X_{\text{ZnS}}^{++} < 0.90$ ) and passed through a maximum about  $X_{\text{ZnS}}^{++} = 0.25$ . A curve of similar shape was also observed at a concentration of 2 N.

The size of spherical pieces of the exchanger was observed under the microscope after allowing them to stand for one hour in solutions containing different ratios

<sup>9</sup> *Techn. Report Office Naval Res. N.R. 057158* (University of Kansas, February, 1949).

<sup>10</sup> Private communication.

<sup>11</sup> Private communication.

<sup>12</sup> Private communication.

of zinc and uranyl ions at the same total concentrations. Although a wide scatter was observed it is clear that the volume of the exchanger also passes through a maximum at roughly the same values of  $X_{Zn^{++}}$  as the maximum obtained in the mass product plot. In solutions of 0.2 N the volume varies by about 5 % over the whole range of  $X_{Zn^{++}}$ .

If one assumes the exchanger to consist of free cations bound by a solid anion in an aqueous phase, volume changes of this order would produce a concentration change which would seem to be quite adequate to account for the observed variation of the mass product, even if it is valid to assume that the thermodynamic equilibrium constant is independent of the ion concentration in the exchanger. Small changes of dilution of the ions in the exchanger will cause large changes in the activity coefficients (since the solutions in the exchanger will be greater than 4 N) and it is unlikely that the ratio of the activity coefficients of the ions would be constant.

**Mr. J. D. H. Strickland** (*Royal Arsenal, Woolwich*) said: May I take this opportunity to mention briefly some of the results that we have recently obtained during an investigation of monovalent ion exchange (chiefly  $K^+$ ,  $NH_4^+$  and  $H^+$  ions) on a sulphonated phenol formaldehyde and a sulphonated cross-linked polystyrene resin. Our findings largely support those of Duncan and Lister and in addition may throw some light on the apparent anomalies in the results of various workers in this field.

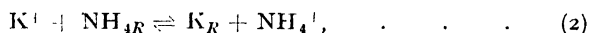
The expression

$$\frac{X_R}{1 - X_R} \times \frac{1 - X_S}{X_S}$$

should be equal to the mass law product  $K$  of an exchange such as:



or



provided that the solutions are sufficiently dilute for activities to be equated to concentrations in the aqueous phase. Nothing definite is known at present concerning activities of the ions in the resin phase and any variations of these are best included in the value for  $K$ .

This expression for  $K$  can be very dependent upon what experimental data are used in its evaluation, especially when  $X_R$  is very small or very large or when the total ionic concentration of the solution is small in comparison with the exchange capacity of the resin. When  $X_R$  is small we find indications of the 'hump' in the plot of  $K$  against  $X_R$  that is shown in Fig. 6 in the paper by Duncan and Lister. We, like they, used an experimental technique which measured  $X_R$  directly and it is assumed that  $X_S$ , etc., are known. These experiments not only measure 'true' exchange but also any adsorption of the exchanging ion (e.g.,  $K^+$ ) that may have taken place. Such adsorption can have a very marked effect on the constant when  $X_R$  is small, even although the adsorption does not amount to more than about 1 % of the exchange capacity of the resin.

When we made measurements of  $K$  at  $X_R = 0.1$  to 0.2, using a technique which measured the change of  $H^+$  or  $NH_4^+$  in solution, the constant again appeared to have exceptionally high values but these could only result from either true exchange or true exchange plus *desorption* of traces of the exchanged ion (e.g.,  $H^+$  or  $NH_4^+$ ) from the resin. When solutions of known composition were equilibrated with a resin and then analyzed we found (at  $X_R = 0.2$ ) that slightly more  $H^+$  or  $NH_4^+$  came off the resin than  $K^+$  went on. In short, during ion exchange both true exchange and the adsorption and desorption of traces of cationic material are probably occurring. Adsorption phenomena of any likely magnitude will generally speaking have little effect on  $K$  but under some circumstances can cause apparently large and contradictory variations, according to the experimental conditions and data used.

For the rest of the  $K - X_R$  plot, we find that with eqn. (2),  $K$  is remarkably constant as shown by Kressman and Kitchener,<sup>13</sup> but that with exchanges

<sup>13</sup> Kressman and Kitchener, *J. Chem. Soc.*, 1949, 1190.

involving hydrogen ions (e.g., eqn. (1)) this is not the case.  $K$  decreases with increasing  $X_R$  (even with the  $\text{Li}^+$  exchange) and with the phenol formaldehyde resin this is particularly marked at  $X_R$  values exceeding 0.9.

We consider that the relative availability of exchange sites on the resin to each of the exchanging ions has bearing on these phenomena and that although with certain exchanges a mass-law product may be valid over most of the isotherm, this will not in general be the case. I fully support the plea of Dr. Duncan for more work in this field and for more agreement upon definitions. As things are, only a very guarded and critical use should be made of so-called thermodynamic equilibrium constants.

**Prof. C. W. Davies** (*Aberystwyth*) said: Before examining special explanations for the variations found in the  $K$  values for exchange equilibria, it is worth looking in more detail into the simplest possible picture of the process, and its consequences. If the resin is to be regarded as a homogeneous phase in Duncan and Lister's experiments, the usual Donnan equations<sup>14</sup> for the distribution of the diffusible ions lead to

$$\left(\frac{a_{\text{Ba}}}{a_{\text{H}}^2}\right)_R = \left(\frac{a_{\text{Ba}}}{a_{\text{H}}^2}\right)_S;$$

on this basis the  $K$  of Duncan and Lister's eqn. (2) would be unity.

If now  $v$  is the volume in litres of resin phase containing one equivalent of exchanging groups, and  $X$  is the fraction of these associated with barium,

$$(a_{\text{Ba}})_R = f'_{\text{Ba}} X^{1/2v},$$

where  $f'_{\text{Ba}}$  is an activity coefficient (volume concentration scale) for the ion in the resin phase. Writing the activity of the hydrogen ion in the same way we get

$$\frac{X}{2(1-X)^2} \cdot \left(\frac{a_{\text{H}}^2}{a_{\text{Ba}}}\right)_S = \frac{f'_{\text{H}}^2}{f'_{\text{Ba}}} \cdot \frac{1}{v}.$$

This equation is similar to Duncan and Lister's eqn. (6) and (10), and clearly a mass-action "constant" is going to vary with changes in  $v$  and in the activity coefficient ratio;  $v$  will undergo large changes with alterations in the extent of swelling of the resin; and the activity coefficient ratio is also a much more important term than has often been supposed, for no matter how dilute the solution may be the ionic strength of the resin phase will be high.

If we traverse Duncan and Lister's Fig. 2 from right to left, the substitution of hydrogen for barium, other things being equal, will almost certainly cause the activity coefficient ratio, and therefore  $K$ , to rise; on the other hand, a substitution of two diffusible H ions for one Ba ion will increase the swelling and cause  $K$  to fall; and in addition to these two opposing effects, the ionic strength of the resin phase will change enormously as we traverse the diagram, causing further unpredictable changes in the activity coefficient ratio. The experimental curves do not therefore appear surprising.

Incidentally, we can get an approximate idea of the magnitude to be expected for  $K$  on this simple picture. At an ionic strength of 3.0, the mean activity coefficient of barium chloride is 0.389,<sup>15</sup> whereas that of 0.01 N HCl in barium chloride solution is 0.823.<sup>16</sup> So for this comparison

$$\gamma_{\text{HCl}}^4 / \gamma_{\text{BaCl}_2}^3 = 7.8.$$

If we take this as a very rough indication of conditions in the resin phase, and as the value of  $1/v$  is about 4 g.-equiv./l. a  $K$  value of around 20 to 30 for this bi-univalent exchange appears quite reasonable.

**Dr. J. F. Duncan** (*A.E.R.E., Harwell*) said: Since the value of  $K_a$  may vary by 50 % or more with concentration and the ratio of the exchanging ions, it becomes difficult to assess the affinity of any ion for a given exchanger without comparing the values of  $K_a$  at corresponding points on the mass-product plot. Values of  $K_a$  quoted in the literature hitherto should therefore be considered as

<sup>14</sup> Cf. Bauman and Eichhorn, *J. Amer. Chem. Soc.*, 1947, **69**, 2830.

<sup>15</sup> Tippetts and Newton, *J. Amer. Chem. Soc.*, 1934, **56**, 1675.

<sup>16</sup> Harned and Geary, *J. Amer. Chem. Soc.*, 1937, **59**, 2032.

approximate only, unless the range and concentration in which  $K_a$  has been determined have been quoted. To avoid further confusion in this matter in the future it seems logical to compare the affinities of ions by quoting the value of  $K_a$  with respect to a constant reference ion (say, hydrogen) at infinite dilution of both ions, with one of the ions at infinite dilution in the exchanger. Thus, for copper we propose to quote the value of  $K_a$  with respect to hydrogen obtained when the concentrations of copper and hydrogen in solution are zero, and  $x_{Cu_2}$  and  $X_{Cu_2} = 0$ . This requires considerable experimental effort to determine and it is possible that at low concentrations adsorptive phenomena become significant, but we consider that this mass product at least has some meaning being unconfused by such variables as concentration, ion ratio and swelling.

**Dr. J. A. Kitchener** (*London*) said: According to the evidence presented by Dr. Duncan and Mr. Lister, no authors have yet found a method of calculating thermodynamic activity ratios for ions in the resin phase; mass-action "constants," by whatever means calculated, are apparently not *accurately* constant over the entire range of an exchange.<sup>17</sup> While sympathizing with the desire which has been expressed to find some standard condition and expression for presenting equilibrium results, I do not favour the choice of "infinite dilution" as the standard state. A non-thermodynamic mass-action product is not fundamentally sounder at infinite dilution than elsewhere, and the very dilute range is experimentally less accessible and practically less useful. For purposes of tabulation, the "constant" for half-exchange might be the most sensible.

It is still true that the mass-action product is *approximately* constant for a limited range. It therefore has some practical utility for that range. For the present, one cannot do better than to present results as some kind of clearly defined "constant" with careful statement of the experimental conditions to which it applies (e.g., a mean over a stated range of resin or solution composition). Where the accuracy of the data justify, the variation of this "constant" with conditions can be recorded as in the paper of Duncan and Lister. Such "constants" are obviously of limited theoretical value, but they still provide the most concise means of recording the experimental results for purposes of comparison and practical calculation.

**Dr. R. W. Richardson** (*Braintree, Essex*) said: The paper of Kunin and Myers has shown the part played by the microstructure of the resin in ion-exchange and I would like to emphasize the importance of this point as it seriously limits the practical usefulness of the method in some cases.

Studying the absorption of the free sulphonic acids of azoic direct cotton dyestuffs (preliminary note submitted for publication elsewhere) I have found that the largest dye acids are rapidly absorbed by Deacidite B to a low final equilibrium figure, this presumably being absorption at the surface of the resin. With a decrease in the molecular size a point is reached at which a slow diffusion into the structure of the resin begins. Progressively smaller dye acids from this point show an increasing rate of absorption to equilibrium figures which rise correspondingly.

If sulphuric acid is taken as completely saturating all the available ion-exchange centres then there exists a decrease by a factor of  $\sim 1000$  in the extent of adsorption in the case of the large Sky Blue FF (Colour Index No. 518) sulphonic acid, a dyestuff which it is assumed reacts only at the surface, 100 for the substituted naphthalene-azonaphthalene and 15 for the benzene-azobenzene molecules.

For the larger dyestuffs, the acidic properties of the sulphonic acid groups were shown to be little affected by the presence of the weakly basic aromatic amino groups. The extent of sulphonation did not appear to alter the relative positions of equilibrium absorption within the series studied; presumably the size of the molecule plays a more significant part in determining the absorption. It is of the utmost importance therefore that new exchange materials be developed with as porous internal structures as are compatible with practical hydraulic properties.

<sup>17</sup> Cf. Kressman and Kitchener, *J. Chem. Soc.*, 1949, 1190.

**Dr. A. Wassermann** (*London*) said: The study of adsorption properties of highly swollen alginate gels, from a thermodynamic and kinetic point of view, is of interest because it indicates suitable methods of an investigation into the adsorption properties of other biologically important materials of similar degree of swelling.<sup>18</sup> The paper by Shepard and Tiselius describes experiments in which proteins are taken up by an inorganic adsorbent. I believe that it is also of importance to measure the adsorption of simple inorganic and organic substances on proteins, under continuous flow conditions, similar to those prevailing in chromatographic analysis. Such experiments involving fully swollen myosin gels of specified nitrogen and potassium or calcium content are carried out by Mrs. M. L. R. Harkness, who could show that this muscle protein is a typical cation-exchange material, the overall effect depending *inter alia* on the relative rate of inter- and intramolecular ionic metatheses.<sup>19</sup> We intend to carry out experiments with a view of finding out (a) whether these cation-exchange reactions at constant pH give rise to alterations in the molecular shape of the adsorbents similar to those observed with fully swollen alginate fibres,<sup>20</sup> and (b) whether these protein gels can be utilized to separate mixtures of certain substance the analysis of which cannot easily be carried out by the conventional chromatographic methods.

**Mr. D. K. Hale and Mr. D. Reichenberg** (*Teddington*) (*communicated*): We would like to draw attention to the important question of the extent to which anions can diffuse into a cation-exchange resin. This has been investigated by Bauman and Eichhorn,<sup>21</sup> who concluded that the concentration of excess electrolyte within the resin was considerably less than the concentration outside, although the results were not in complete agreement with the simple Donnan theory. We are indebted to Dr. W. C. Bauman for pointing out to us that the increase in rate of exchange observed with increasing hydroxyl ion concentration might be accounted for by the diffusion of hydroxyl ions into the resin and the neutralization of hydrogen ions within the resin phase. We do not consider our own experiments decisive on this point but the agreement between the results obtained with N NaOH and 2 N NaOH solutions (Fig. 8) suggests that, with the resin we used, a limiting rate of exchange is reached at a hydroxyl ion concentration of M or below. Whether this limit is due to the elimination of hydrogen ion film diffusion as we have suggested, or to some other cause remains an open question. The extent of diffusion of excess electrolyte into the resin phase must also be considered in the determination and interpretation of affinity constants.

**Dr. A. Wassermann** (*London*) said: \* In collaboration with Mr. Mongar a microcalorimetric method has been developed<sup>22</sup> which enables an estimation to be made of heat effects accompanying the interaction between filter paper strips, fully swollen with water (the dry weight of the paper was about 6 mg.) and 0.02–0.04 cm.<sup>3</sup> of the electrolyte solution. It could be established that the heat of swelling of the filter paper in water is markedly different from the heat of swelling of the same paper<sup>23</sup> in the electrolyte solution, there being no irreversible reaction between the cellulosic material and the salts.

<sup>18</sup> Cf. Wassermann, *Nature*, 1946, **158**, 271; 1948, **161**, 562. *Ann. Botany*, 1949, **13**, 79.

<sup>19</sup> A description of this work will be given in the near future, preliminary reports having been submitted on 21st September, 1949, to the Secretary of the Department of Scientific and Industrial Research.

<sup>20</sup> Mongar and Wassermann, *Nature*, 1947, **159**, 746. MacArthur, Mongar and Wassermann, *Nature*, 1949, **164**, 110.

<sup>21</sup> Bauman and Eichhorn, *J. Amer. Chem. Soc.*, 1947, **69**, 2830.

<sup>22</sup> The technique is a modification of that used by Hill (see e.g., *Proc. Roy. Soc. B*, 1938, **126**, 136) for a study of heat effects accompanying electrically stimulated contractions of frog sartorii.

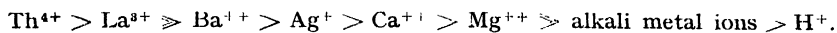
<sup>23</sup> The carboxyl value of the paper was so small that ion exchange did not occur to any appreciable extent.

\* In connection with a remark by Prof. Tiselius relating to filter-paper partition chromatography.

**Dr. E. Heymann** (*Melbourne, Australia*) (*communicated*): The information obtained from investigations of equilibria between cation exchange resins and electrolytes in solution can be supplemented by investigation of the electric conductivity of swollen plugs of resin containing various cations.<sup>24</sup> We use a conductivity cell in which the resin is packed between perforated platinum electrodes. This cell allows of replacement of one ionic species by another in the same plug. We investigate sulphonic acid resins.

The hydrogen resin and the alkali metal resins are fairly good conductors of electricity, of the same order as dilute solutions of strong electrolytes. The conductivity of these resins is roughly proportional to the mobility of the respective cations in water. This suggests that these cations are weakly bound in the resin.

Resins containing bivalent cations show much lower conductivities than the first group, and there is no proportionality between resin conductivity and mobility of the cation in water. Resins containing cations of higher valency are poor conductors. Obviously these ions are bound more strongly than the univalent ones. Our investigations suggest the following series of strength of binding in the resin Amberlite IR-100:



This series is similar to that obtained by Boyd, Schubert and Adamson<sup>25</sup> from equilibrium studies.

It is probable that the conductivity of the swollen resin plugs is not solely determined by the strength of binding, but different degrees of swelling of resins containing various cations may also have an effect. These effects are under investigation at present.

**Dr. D. J. Crisp** (*I.C.I. Paints Division*) (*communicated*): It is worth drawing attention to the phenomenon of exchange in ionized surface films, which represent in certain respects the most simple of ion-exchange systems. Hitherto when experiments have been described in which the ionic composition of the substrate below the film has been changed, emphasis has been placed upon the resulting changes in surface pressure and surface potential, rather than upon the exchange of small quantities of ions between the monolayer and the solution. As a result, processes which are strictly ion exchanges have not been presented from this standpoint, but have been termed "adsorption" (in the case of simple and polyvalent ions) or "penetration" (with organic ions) as though the addition or entry of a new ionic species were the only process taking place.

The use of charged monolayers to elucidate details of ion-exchange phenomena may prove very useful by making possible the measurement of certain characteristics of the system which are not readily observed with solid ion exchangers. For example, the phase-boundary potential indicates that as the concentration of one ionic species of opposite sign from that of the monolayer is increased, there is at first a simple exchange of ions followed by a reduction in the thickness of the double layer; the relative concentrations at which exchange occurs with different monovalent organic ions is simply related to the van de Waals' interaction of the ion with the film.<sup>26</sup> The advantages of using such systems may be summarized thus:

- (a) Virtually instantaneous equilibration between the monolayer and solution.
- (b) Exchanges between the film and solution do not sensibly affect the concentration of the latter.
- (c) The monolayer represents a two-dimensionally homogeneous exchange system in which all sites are equivalent to one another.
- (d) The capacity of the film may be regulated by altering its specific area.
- (e) Changes in surface pressure or surface area resulting from ion exchange are two-dimensional analogues of swelling phenomena in solid exchangers, and are easily measured.
- (f) The phase-boundary potential can be determined.

<sup>24</sup> Heymann and O'Donnell, *J. Colloid Sci.*, 1949.

<sup>25</sup> Boyd, Schubert, and Adamson, *J. Amer. Chem. Soc.*, 1947, **69**, 2818.

<sup>26</sup> Crisp, *Surface Chemistry* (Butterworths, 1949), p. 65.

As a useful cation-exchange monolayer, the  $\alpha$ -halogenated fatty acids with 16-18 carbon atoms may be recommended. These are well ionized in neutral solution—as distinct from the unsubstituted fatty acids—and are not excessively soluble as are the sulphonic acids—and therefore produce well-ionized but quite stable monolayers.

**Prof. A. Tiselius** (*Uppsala*) said: It seems to me that the combination of partition and displacement methods applied by Dr. Levi offers very great promise on account of its extremely high specificity. The losses due to irreversible adsorption (for example on charcoal) have somewhat limited the use of the displacement development so far, but one may perhaps hope that such losses would be considerably less on a partition column.

A similar procedure applied in filter-paper partition chromatography might offer a means of making this method quantitative, as in displacement development, so that the length of a zone is proportional to the amount of substance which it contains.

It also seems to me that, as filter paper originally has not been manufactured for chromatographic purposes, much improvement could be hoped for by close collaboration with the manufacturers, especially as regards homogeneity, purity and possibly also elimination of the causes of losses due to tailing or irreversible adsorption.

**Dr. J. Boldingh** (*Zwijndrecht, Holland*) said: With regard to the rubber-coated paper strips for fatty-acid analyses additional information about the quantitative separation of the even numbered saturated fatty acids may be given. A column of powdered rubber (Mealorub) which has been swollen with benzene is used; the mobile phase consists of a mixture of methanol-acetone (3/1) with varying proportions of water. By decreasing the water content of the mobile phase at the right moment separation of the  $C_6$ - $C_{18}$  series has been made possible with a recovery of 95 % to 100 %. More recent experiments with another solvent combination have made it possible even to separate mixtures containing the series  $C_6$ - $C_{24}$  in one straight run. With a column of 15-20 cm. length and a diameter of 1.2 cm., a mixture containing 2-10 mg. of each acid can be analyzed. Mealorub may be obtained from the "Rubber Stichting" at Delft (Holland). Details will be published later.

**Prof. R. M. Barrer** (*Aberdeen*) said: Crystalline silicates exist which provide regular networks of channels with diameters no bigger than those of quite small molecules. Such crystals could act as sieves and might bring about separations of molecular species by occluding the smaller molecules while acting as non-sorbents towards other larger or wrongly shaped molecules. Among zeolites there are a number of species which are capable of producing molecular sieve separations in this manner, and I have in my paper indicated some of their properties which are relevant to the separating of molecular mixtures.

When a gaseous mixture is passed through a porous zeolitic bed one may have Poiseuille flow or Knudsen flow in macroscopic spaces between crystal grains together with intracrystalline diffusion into the individual crystallites. The rate of transpiration must be adjusted so that sorbable species may be occluded during the time of transit, and so removed from the gas stream. In the region of molecular streaming (or Knudsen flow) the diffusion coefficient in absence of sorption effects in a cylindrical macroscopic capillary is

$$D_1 = -\frac{4r^2}{3\sqrt{\frac{\pi m}{2kT}}}, \quad (1)$$

while the diffusion coefficient of sorbate into the individual crystallites is

$$D_2 = D_0 e^{-E/RT}. \quad (2)$$

In many cases  $E$  is small and the condition for a good separation by gaseous chromatography may be fulfilled (i.e., that rate of uptake inside crystallites is at least comparable in velocity with the time of transit through the powder).

Either occlusion or adsorption, by immobilizing a proportion of the molecules, can prolong the time of transit of one species compared with another non-sorbed

molecule. In the low-pressure range (molecular streaming) one has for the sorbable component

$$\frac{\partial c}{\partial t} = D_1 \frac{\partial^2 c}{\partial x^2} - \frac{\partial S}{\partial t} - \frac{\partial S_1}{\partial t} \quad . \quad . \quad . \quad (3)$$

where  $S$  and  $S_1$  are the amounts adsorbed and occluded respectively per unit volume of the sorbent bed. When both adsorption and occlusion are rapid enough, and at low pressures,

$$\left. \begin{aligned} S &= Ck \text{ (linear adsorption isotherm) } \\ S_1 &= Ck_1 \text{ ( " occlusion " ) } \end{aligned} \right\} \quad . \quad . \quad . \quad (4)$$

and substituting in eqn. (3) gives

$$\frac{\partial c}{\partial t} = \frac{D_1}{(1 + k + k_1)} \frac{\partial^2 c}{\partial x^2} \quad . \quad . \quad . \quad (5)$$

and the occlusion process reduces the diffusion coefficient of the sorbable component in the ratio  $1/(1 + k + k_1)$ . In such a system good separations may occur should  $k$  and  $k_1$  be large enough. In zeolites this is often the case. Moreover in the steady state of flow

$$P = \frac{D_1(c_1 - c_2)}{(1 + k + k_1)l} \quad (l = \text{bed thickness, } c_1, c_2 = \text{ingoing and outgoing concentrations}),$$

and the permeability  $P$  is no longer proportional to  $\frac{1}{\sqrt{m}}$  for a series of such gases as in Knudsen flow in absence of sorption effects.

**Dr. R. L. M. Synge** (*Rowett Res. Inst.*) said: Without wishing to discuss the mechanisms proposed by Weiss for the interactions which he has observed on charcoals doped in various ways, I would like to emphasize that the modification of the adsorptive properties of charcoal for chromatographic purposes by adsorbing on it substances not significantly eluted by the developing solvent deserves further study. Recent work at Uppsala<sup>27</sup> showed that by doping charcoal with stearic acid a product was obtained which, though of lower adsorptive capacity, had more nearly linear isotherms with amino acids and peptides in aqueous solution, and showed greater specificity in separating aromatic from aliphatic compounds.

**Mr. A. Stewart** (*Grangemouth*) said: The slurry method for packing columns with a fairly granular adsorbent is preferred as entrapped air escapes in the initial mixing and the columns are substantially free from air bubbles. Closest packing is obtained by tapping uniformly, preferably stopping and restarting the solvent flow at the same time. These columns show greater band movement in the middle than at the periphery. Dry packing of such adsorbents may give columns containing small air bubbles which cause the movement at the band edges to be somewhat irregular; the tamped dry-packed columns also have a greater resistance to solvent flow.

**Mr. R. H. S. Robertson** (*Glasgow*) (*communicated*): Although in chromatography it is usual to separate a mixture of dissolved substances by means of a standard adsorbent, the opposite procedure of using a standard mixture of solutes to examine an unknown adsorbent has been found to be a useful diagnostic test.

Half a gram or less of powder is squashed into a smooth concave surface with a spoon, and three drops of the standard mixed solution are rapidly poured into the basin so formed. The development of the colour rings is observed and recorded. Standard solutions can be made up to give an indication of pH, of chemical reactions and of physical adsorption, according to the nature of the substances in the powder. The method has proved of value in the examination of diatomites, cosmetic powders and clays.

<sup>27</sup> Synge and Tiselius, *Acta Chem. Scand.*, 1949, **3**, 231.



## SUMMARIZING PAPER

By R. L. M. SYNGE

*Received 6th October, 1949*

In this first section of the Discussion we were expected to deal with the basic principles of chromatography, including the mathematics and the physical chemistry. The original invitations to contributors were distributed in the hope of obtaining reviews covering the whole field more or less systematically. However, many of the contributions received and printed are accounts of original work. This is an indication of the active and healthy development which the subject is undergoing. In our discussion of the printed communications we have ranged over most of the ground to be covered, though not in any orderly manner. This makes the task of summing up the Discussion both more difficult and more necessary. Perhaps it can conveniently be done under four heads, as follows:

- (1) Mathematical treatment ;
- (2) Operating procedures ;
- (3) Techniques for analysis and visualization ;
- (4) Physical and chemical mechanisms of the equilibria determining separations.

(1) **Mathematical Treatment.**—Those who have engaged in mathematical analysis of chromatographic operations have not always stated clearly what simplifications and assumptions they have made and at what points in their argument physical principles are left behind and purely mathematical manipulations embarked on. These errors of presentation have made the subject a particularly difficult one to disentangle for those of us who are not mathematicians, and have helped to encourage a widespread but erroneous idea that there are a number of "rival theories" of chromatography. In fact, the general nature of the relevant processes going on at different points in a chromatographic column—diffusion, streamline flow, adsorption, desorption, transfer between phases, ionization and so forth—is well known to all of us, and in most cases these processes obey laws capable of expression in rather simple mathematical terms. However, the multiplicity of points in space and instants in time that have to be considered makes the full mathematical treatment impossibly complicated, and pending developments in mathematical technique it is necessary to make simplifying assumptions and to consider particularly simple operating conditions. Two main types of assumption have proved useful. One is the "theoretical plate" concept of conventional counter-current theory, where the operation of all such factors as diffusion, slowness in attaining equilibrium and microscopic irregularities of flow is abstracted into the simple unit H.E.T.P. (height equivalent to a theoretical plate). The behaviour of substances having linear and mutually independent distribution isotherms between stationary and moving phases is then capable of simple treatment. The other assumption is to postulate instantaneous local equilibrium, that is, to put  $H.E.T.P. = 0$ , and to dismiss the processes abstracted into that concept as "disturbing factors." The behaviour of substances with non-linear distribution isotherms can then be treated, as can the interaction

of such substances. The treatment is particularly simple for the cases of frontal analysis and of completed displacement development, as shown in Prof. Claesson's contribution. Of course it is not fortuitous that the most effective chromatographic operating procedures lend themselves to particularly simple mathematical treatments, since in them steps are taken to minimize effects of processes that do not contribute to the desired result. Even in the intermediate cases where mathematical treatment becomes difficult, it is often possible to visualize qualitatively what the effect of a change in working conditions will be, and one can also check in practice what factors are contributing to the result by varying one factor at a time, maintaining other conditions constant. In fact, such "simple-minded" approaches have probably contributed more to the understanding of chromatography than have the more intricate mathematical analyses.

(2) **Operating Procedures.**—Prof. Tiselius has distinguished three main methods by which chromatograms may be developed; these are:

(a) **FRONTAL ANALYSIS**, where the original mixed solution is passed continuously through the column.

(b) **ELUTION DEVELOPMENT**, where the original mixed solution is placed on the column in small volume and washed through with pure solvent.

(c) **DISPLACEMENT DEVELOPMENT**, where the original mixed solution is placed on the column in small volume and washed through with a solution of some displacing agent.

There would be advantages in adopting this nomenclature, and using it consistently. (The symbols used in connection with chromatography could also with advantage be standardized. It is particularly confusing that the symbol for band-rates  $R$  adopted by LeRosen<sup>1</sup> and used by some subsequent authors is not defined in the same way as  $R$  of Martin and Synge<sup>2</sup> but is the same as  $R_f$  of Consden, Gordon and Martin.<sup>3</sup>)

As regards these operating procedures two points seem to require emphasizing. One is the special value of frontal analysis for analyzing unknown mixtures of substances having unknown properties. The other is that for displacement development to be effective the necessary condition is not that the substances undergoing separation should exhibit curved distribution isotherms, but that they should compete with and displace one another on the column. Should this not be so, no amount of curvature of the isotherms will lead to effective separations by displacement development.

It is good that Prof. Claesson has raised the question of the analogy between chromatographic and electrophoretic processes. Consideration of the electrical migration of substances in a stirred many-compartment diaphragm cell, where the pH and conductivity of each compartment is maintained constant and all the diaphragms are thin and of the same material, shows that substances will migrate from one compartment to the next at rates determined by their relative transference through the membrane, the amount of electro-endosmosis and so forth. However, the amount leaving a given compartment will always be directly proportional to the amount in that compartment. Thus conventional counter-current theory can be applied to such a set of compartments—the relative transference and electro-endosmosis of the substances determining the enrichment factors and the linear relationship between amount present and amount migrating giving the mathematical equivalent of a linear distribution

<sup>1</sup> LeRosen, *J. Amer. Chem. Soc.*, 1942, **64**, 1905.

<sup>2</sup> Martin and Synge, *Biochem. J.*, 1941, **35**, 1358.

<sup>3</sup> Consden, Gordon and Martin, *Biochem. J.*, 1944, **38**, 224.

isotherm. Each compartment corresponds to one theoretical plate, and substances initially present in one compartment will distribute themselves as they migrate approximately according to the Gaussian curve. Migration in a jelly can then be viewed as the juxtaposition of a number of small compartments separated by diaphragms. The enrichment factor between two migrating substances is then simply the ratio of their net mobilities, after allowing for electro-endosmosis, and the H.E.T.P. is determined by the diffusion constants for the substances in the jelly. In electrophoresis in free solution, H.E.T.P. is likewise an expression of the diffusion constant.

This way of looking at electrophoretic phenomena clears up a point that had puzzled me for some time—the justification of the use of the H.E.T.P. concept by Brewer, Madorsky and colleagues<sup>4-7</sup> for the electrical migration of ions in a tube packed with sand against a stream of water. Westhaver<sup>7</sup> gives a detailed analysis of the role of diffusion and lack of uniformity of flow of solvent in the pores in determining the H.E.T.P. Much of this treatment can be used directly for considering factors determining H.E.T.P. in ordinary chromatograms. Dr. Klinkenberg has already emphasized in discussion the importance of flow mechanisms in determining column efficiency.

It is very valuable that electrical migration is compatible technically with chromatography. Some obvious applications are :

- (a) The interpretation of adsorption effects in jelly ionophoresis.
- (b) The use of electro-endosmosis for moving liquids through a jelly and observing molecular sieve effects, as mentioned by Prof. Tiselius in his Introductory Paper.
- (c) The combination, in suitable instances, of chromatographic and electrical procedures, as done by Strain,<sup>8</sup> to economize the number of operations in a separation.

There are certain operating procedures that do not at first sight fall into any of the categories so far discussed. One of these is the stationary type of chromatogram, such as those on alumina described by Prof. Flood or with penicillins on alkali-impregnated silica, described by Catch, Cook and Heilbron.<sup>9</sup> These are really frontal analyses, but the mutual competition of the solutes for stoichiometric combination in the stationary phase is so intense that what is virtually a displacement chromatogram results, and the different band lengths may be used for quantitative analysis just as in displacement development.

Another, and very important, operating procedure is development with successive changes of solvent. Very valuable results have been obtained with this procedure from the earliest days of chromatography and numerous examples of its use are given in this Discussion. It may perhaps be regarded as a form of displacement development where the solvent or eluting agent displaces some components, but not others, of the mixture being separated. This leads to the introduction of a spatial gap between the components in question, which facilitates their separation from each other. They can usually be separated from the solvent or eluting agent quite easily, taking advantage of differences in physical or chemical properties such as volatility. In simple displacement development there is no spatial gap between successive components, and this leads to technical difficulties in cutting.

<sup>4</sup> Brewer, Madorsky and Westhaver, *Science*, 1946, **104**, 156.

<sup>5</sup> Brewer *et al.*, *J. Res. Nat. Bur. Stand.*, 1947, **38**, 137.

<sup>6</sup> Madorsky and Straus, *J. Res. Nat. Bur. Stand.*, 1947, **38**, 185.

<sup>7</sup> Westhaver, *J. Res. Nat. Bur. Stand.*, 1947, **38**, 169.

<sup>8</sup> Strain, *J. Amer. Chem. Soc.*, 1939, **61**, 1292.

<sup>9</sup> Catch, Cook and Heilbron, *Nature*, 1942, **150**, 633.

Chromatograms in which there is a continuous change in operating conditions along the length of the column (such as would be caused by evaporation from flowing mixtures of solvents) do not seem to have received much consideration, though I have more than once heard Prof. Tiselius discuss their possibilities. There seems no reason for expecting such systems to be useful when completeness of separation is the main consideration. However, one can imagine a dilute mixed solution entering such a chromatogram continuously, the result being an accumulation of different solutes at different points. Such systems must be of common occurrence in nature, responsible for some of the concentrations of substances observed in rocks and soils, and possibly capable of being employed in technology.

(3) **Techniques for Analysis and Visualization.**—This subject is, in a sense, a subsidiary aspect of chromatography. One can note a general tendency away from the practice of extruding and cutting up chromatograms, and in favour of observation and analysis of the effluent. When dealing with unknown mixtures, there is a great deal to be said for taking numerous arbitrary fractions, and analyzing these retrospectively, since it may not be apparent at the start what properties should be taken into account in making the cuts, whereas if the fractions are small enough they can later be pooled in the groups found most suitable. Chromatographers owe a debt to Dr. Stein and Dr. Moore<sup>10</sup> for introducing an automatic fraction-cutting technique, which enormously reduces the labour involved.

Numerous authors in this Discussion have used radioactive substances as markers on chromatograms, and this procedure certainly has very general significance for observational purposes. Prof. Brockmann's new fluorescence procedures also deserve further study.

(4) **Physical and Chemical Mechanisms of the Equilibria Determining Separations.**—Several contributors have described kinetic studies in this Discussion, and it has been suggested that differences in kinetics of approach to equilibrium could be utilized for separations in addition to differences in equilibrium. This is true, but it should be emphasized that such separations would not be chromatography in the usual sense but would require a quite different type of apparatus, perhaps similar to that used by Signer and colleagues.<sup>11</sup>

The exploration of different mechanisms determining equilibrium distributions in chromatographic systems, and of the specificity of these mechanisms for different substances, is at present in a phase of rapid development and is daily yielding valuable results. Prof. Tiselius in introducing the Discussion dealt with this topic at some length and there is little to add. The contributions of Prof. Barrer and of Dr. Myers and Dr. Kunin suggest that, before long, molecular-sieve properties will be utilized for chromatographic separations of compounds extending over a fair range of molecular sizes. There seem also to be possibilities, so far little explored, for increasing the specificity of adsorption processes. In this connection, the proposal of Dr. Wassermann, mentioned in discussion, to study the adsorptive properties of proteins has special interest. In this group of substances we know, from the data of serology and enzymology, that specificity of adsorption is capable of being very great. There seems also every reason for hoping that chromatography will prove a useful technique for effecting separations of high molecular substances.

In concluding, I would like to stress how much we can hope to learn by further detailed studies of the ultramicroscopic structure and properties

<sup>10</sup> Stein and Moore, *J. Biol. Chem.*, 1948, **176**, 337.

<sup>11</sup> Signer, Hänni, Koestler, Rottenberg and v. Tavel, *Helv. chim. Acta*, 1946, **29**, 1984.

of the substances used for making chromatographic columns. Until we have much more accurate ideas on this subject, the chromatographic art will remain largely empirical, and our interpretations speculative. We shall learn much by trying to interpret in physico-chemical terms the practical achievements of chromatography that will be presented in the second half of this Discussion. However, other techniques than chromatography will also be required for developing this much-needed body of knowledge.

*Rowett Research Institute,  
Bucksburn,  
Aberdeenshire.*

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## II. APPLICATIONS

### Introductory Paper

BY F. A. ROBINSON

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My first impulse on being invited to introduce the section dealing with the applications of chromatography was to relate some of the more important advances that have resulted from the use of this new tool. On reflection, however, I decided that it would be more profitable to spend the few minutes at my disposal in attempting to assess the suitability for different purposes of the various forms of chromatography now available. I have been encouraged to follow this line of thought because at each of our sessions several speakers have asked whether there is any method of predicting the most favourable conditions to use in any given circumstances. I therefore wondered if it would be possible to obtain any guidance from an analysis of the data presented at this meeting; for I think we can take the contributions to this Discussion as fairly representative of trends in recent research in this field.

We have already heard much about the three main branches of chromatography—adsorption, partition and ion-exchange chromatography—and the three main methods of development—displacement development, elution development and frontal analysis—the relative advantages and disadvantages of which have been so admirably summarized by Dr. Synge. Now suppose someone without previous experience of chromatography were asked to separate two or three substances chromatographically, how would he proceed? How would he find his way amongst the “enormous number of alternatives possible,” as I believe Prof. Tiselius expressed it? In the early days when I prepared my first chromatogram the procedure was simple; one used alumina or lime or magnesia, and most probably it would have been an unsuitable grade at that, simply because no other was available. Nowadays, however, the position is very different; there is a choice of three fundamentally different techniques and within each of these techniques a fairly wide choice of column materials, eluting agents and methods of manipulation. Which of the possible permutations and combinations should one select?

In the first place, of course, the answer will depend on how much or how little the experimenter knows about the properties of his substances. He

may know nothing, in which event he must try various techniques in turn. But he may know that his substances are neutral, acidic or basic; he may know something of their relative strengths or partition coefficients or even something about their adsorption isotherms. This is unusual, and more often than not he will only have a very general idea of their chemical behaviour. Even this, as I hope to show, should help in making a choice of techniques.

Now the papers prepared for this Discussion refer to the use of adsorption chromatography for the separation of the following substances—metals, hydrocarbons, fats and fatty acids, steroids, high polymers, proteins and carotenoids. The significant point about this list to my mind is that with the exception of cations, which are separated by ion exchange anyway and not by true adsorption, and fatty acids, these substances consist solely of organic compounds free from functional groups, suggesting that these are the substances for which adsorption chromatography is nowadays considered most appropriate.

The list of substances for the separation of which partition chromatography has been used is rather different. True, it includes metal ions and fatty acids, but for the rest it comprises sugars and sugar derivatives, amino acids and peptides, penicillins and vitamin B<sub>12</sub>, all of which are organic compounds with well-defined polar groups.

The list of substances to which ion-exchange chromatography has been applied has much in common with the last list. It includes anions and cations, penicillins, purine bases, nucleic acids and amino acids. This means, of course, that ion-exchange chromatography is most appropriate for the separation of inorganic and organic ions.

I would therefore suggest that the chances are that adsorption chromatography is likely to give the best results with non-polar molecules, partition chromatography with organic compounds containing polar groups and ion-exchange chromatography with inorganic ions and organic acids and bases.

Like most sweeping generalizations, mine has many exceptions—I am sure that each of you will be able to think of several—and I will immediately qualify my statement by suggesting that adsorption chromatography offers many advantages compared with the newer techniques and may retain this advantage for some time to come.

In the first place it is a much older technique and therefore more is known about it. Useful empirical rules have been prepared that simplify the task of selecting the most suitable adsorbents and eluents; these are to be found in the text-books of chromatography. In the second place, adsorption chromatography is the method that lends itself most readily to the application of frontal analysis, which gives the maximum of information about the qualitative nature of a mixture, and displacement development, which gives the maximum amount of quantitative information. However, we must not lose sight of the fact which has emerged during the Discussion that displacement development can be applied both to partition chromatography and to ion-exchange chromatography, so that this particular advantage may not long remain peculiar to adsorption chromatography.

One further reason for the retention of adsorption chromatography for the separation of organic compounds containing functional groups is that colourless substances of this type can often be converted into coloured or fluorescent derivatives that are readily located on the column. Another factor that may for the time being favour the retention of adsorption chromatography in fields where one might expect it to be superseded by partition and ion-exchange chromatography is that at present it is possible to obtain standardized aluminas and, in some degree, standardized carbons,

but it is not yet possible to obtain standardized carriers for holding the static phase in partition chromatography; satisfactory grades of silica gel are notoriously difficult to prepare and both starch and filter papers are variable substances, although not everyone may subscribe to this view. Nor can the present range of ion-exchange materials be regarded as entirely satisfactory, although there are indications that new and, one hopes, better products may soon become available.

I think that in the past workers have been negligent in failing to publish fuller details of the properties of the chromatographic agents they have used, and it is no use bemoaning the fact that manufacturers will not supply suitable reagents unless potential users will go to a little trouble in defining in unambiguous terms the properties they regard as desirable. Ideally, a specification should be prepared for each chromatographic agent, whether required for adsorption, partition or ion exchange, and it might then be possible for manufacturers to supply standardized materials that will give reproducible results not only in the hands of the same workers, but also in different laboratories.

I must confess that I find myself unable to fit Mr. G. Robinson's method into my classification and I am not sure whether to regard the 8-hydroxy-quinoline column as a special example of adsorption chromatography in which separation depends on differences in the adsorption affinities of oxine salts for oxine, or as an example of partition chromatography in which separation depends on the relative solubilities of oxine salts in water, or as the first example of an entirely new type of technique to be called perhaps 'reagent chromatography,' in which separations are effected by differences in the rates of reaction with a selective reagent. It would be interesting to hear of other types; I know only of this and of violuric acid and 5-oxo-4-oximino-3-phenyl-isoxazoline, but the principle should clearly be capable of further extension.

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## II(A). INORGANIC

### NATURE AND APPLICATIONS OF INORGANIC ALUMINA CHROMATOGRAPHY \*

BY GEORGE-MARIA SCHWAB

*Received 21st June, 1949*

The optical spectra of cations adsorbed by sodium exchange on alumina confirm the view, derived from chemical evidence, that the adsorption zones contain basic double aluminates. Inorganic chromatographic analysis is essentially improved by combining it with the usual group separation reactions. The microchemical sensitivity of chromatographic cation detection is about equal to that of spot tests. The chromatographic separation of the platinum metals is described.

\* Contribution from the Department of Inorganic, Physical and Catalytic Chemistry of the Institute Nicolaos Canellopoulos, Piræus, Greece.

The chromatographic adsorption method of Tswett was first applied to the analytical separation of inorganic cations by Schwab and Jockers<sup>1</sup> and of inorganic anions by Schwab and Dattler.<sup>2</sup> These authors, using alumina containing exchangeable sodium for the cation separations and acidified alumina containing exchangeable anions for the anion separations, established the basic principles of ion-exchange chromatography which in the following years acquired so great an importance for amino acid separations<sup>3</sup> and, after the introduction of organic polymer ion exchangers, for the separation of rare earths and pile products.<sup>4</sup> In the present communication, some new progress in inorganic alumina chromatography of practical interest will be summarized. It has been published in periodicals rather difficult to obtain at present.

**The Nature of the Ion Adsorption.**—In the first publications<sup>1,2</sup> it was stated that the adsorption of ions on alumina columns takes place by the displacement of ions of equal sign contained in the alumina surface, i.e., sodium by cations, and nitrate or chloride by anions. The specificity of the adsorption, which is manifested in a reproducible adsorption series, together with the fact that adsorbed cations are accompanied by a certain amount of anions, lead to the conclusion that the adsorption complex, especially in cation chromatography, is a basic double salt, e.g., a basic aluminate-sulphate. Doubts about this result have been expressed by Siewert and Jungnickel<sup>5</sup> who assumed that the adsorption and the desorption series could be explained by the precipitation of carbonates on the column by the sodium carbonate present. However, this opinion, based on an incorrect interpretation of the solubility product and of titration results, has been revised in a joint publication<sup>6</sup> in favour of the original theory.

This latter has been directly substantiated by Schwab and Issidoridis<sup>7</sup> by the measurement of the light absorption of the adsorbed ions by reflected light. It was shown that with cupric ions a new absorption band at 602 m $\mu$  appears on adsorption lower than that of the hydroxide and characteristic of Cu-O bonds. With chromic ions a shift of the 575 m $\mu$  band to longer wavelength occurs, dependent on the anion present, this being less with chromite but greater with the hydroxide. CrAlO<sub>3</sub> shows the opposite shift. In nickel adsorption the 680 m $\mu$  band is shifted to shorter waves, the displacement being less than with the hydroxide. The uranyl ion gives a shift of the 660 m $\mu$  band towards the violet end, this being less than with sodium uranate. All these facts show that the state of the adsorbed layers is intermediate between the free hydrated ion and hydroxide, and this is compatible with the assumption of basic salts for copper, nickel and uranium, and of a chromite-like structure for the strongly adsorbed chromium.

**Chromatography in Qualitative Analysis.**—It had been found that a full qualitative cation analysis by alumina chromatography is rendered difficult by the coincidence of certain zones and the chemical interference of others (Ag-Mn, As-Fe, Cu-Al). Schwab and Ghosh<sup>8</sup> found that these shortcomings could be overcome by combining the usual analytical group separations with a chromatographic identification within each group.

<sup>1</sup> Schwab and Jockers, *Angew. Chem.*, 1937, **50**, 646.

<sup>2</sup> Schwab and Dattler, *Angew. Chem.*, 1937, **50**, 691.

<sup>3</sup> Kuhn and Wieland, *Ber.*, 1940, **73**, 968.

<sup>4</sup> A series of papers in *J. Amer. Chem. Soc.*, 1947, **69**.

<sup>5</sup> Siewert and Jungnickel, *Ber.*, 1943, **76**, 210.

<sup>6</sup> Schwab, Siewert and Jungnickel, *Z. anorg. Chem.*, 1944, **252**, 321.

<sup>7</sup> Schwab and Issidoridis, *Z. physik. Chem. B*, 1942, **53**, 1.

<sup>8</sup> Schwab and Ghosh, *Angew. Chem.*, 1939, **52**, 389.



Thus, the hydrochloric acid group  $\text{Pb}^{++}$ ,  $\text{Ag}^+$ ,  $\text{Tl}^+$  is accessible to such identification. From nitrate solutions, lead is adsorbed at the top, silver in the middle and thallium at the bottom of the column. With potassium chromate as developer, the lead zone turns yellow, the silver zone red and the thallium zone yellow. With ammonium sulphide the lead zone is black, the silver zone grey and a black thallium zone is washed down the column. Mercurous ion must not be present, as mercury is generally unfit for chromatography.

The hydrogen sulphide group is also well split into zones, provided that  $\text{Hg}^{++}$  and  $\text{Sn}^{++}$  are absent and that the solution, the column and the washing water contain some tartaric acid. With a hydrogen sulphide solution as developer one obtains at the top an orange antimony zone, and below a yellow arsenic zone, a black lead zone, a brownish-black copper zone and a yellow cadmium zone.

TABLE I

No.	Cation	Reagent or Developer	Colour of Zone	Sensitivity in $\gamma$	
				Spot-test	Chromatographic
1	$\text{Fe}^{+++}$	$\text{Fe}(\text{CN})_6^{4-}$	Blue	0.04	0.01
2	$\text{Cu}^{++}$	$\text{Fe}(\text{CN})_6^{4-}$	Brown-red	—	0.02
	$\text{Cu}^{++}$	$\text{H}_2\text{S}$	Brown-black	—	0.4
	$\text{Cu}^{++}$	Thio-oxamide + $\text{NH}_3$	Black	0.006	0.005
3	$\text{Co}^{++}$	$\alpha$ -Nitroso- $\beta$ -naphthol	Brown	0.05	0.06
	$\text{Co}^{++}$	Thio-oxamide + $\text{NH}_3$	Brown-black	0.03	0.04
	$\text{Co}^{++}$	$(\text{NH}_4)_2\text{S}$	Brown-black	—	0.2
4	$\text{Ni}^{++}$	$(\text{NH}_4)_2\text{S}$	Green-black	—	0.3
	$\text{Ni}^{++}$	Thio-oxamide + ammonia	Violet	0.012	0.01
5	$\text{Tl}^+$	$(\text{NH}_4)_2\text{S}$	Black	—	1.0
	$\text{Tl}^+$	$\text{KI}$	Yellow	0.6	0.12
6	$\text{UO}_2^{++}$	$\text{Fe}(\text{CN})_6^{4-}$	Faint yellow	0.92	0.45
7	$\text{Ag}^+$	$(\text{NH}_4)_2\text{S}$	Black	—	1.0
	$\text{Ag}^+$	<i>p</i> -Dimethylamino-benzylidene rhodamine	Violet	0.02	0.02
	$\text{Ag}^+$	$\text{Mn}^{++} + \text{OH}^-$	Black	2.0	0.11
8	$\text{Pb}^{++}$	$(\text{NH}_4)_2\text{S}$	Dark black	—	0.54
	$\text{Pb}^{++}$	$\text{CrO}_4^{2-}$	Yellow	—	0.54
9	$\text{Cd}^{++}$	$(\text{NH}_4)_2\text{S}$	Yellow	—	0.54

The most important possibility is the separation of the ammonium sulphide group in one operation. With ammonia as developer one obtains a brown iron zone, a green chromium zone, a yellow uranyl zone, a white zinc zone, a rose-colour cobalt zone, a blue-green nickel zone and a nearly colourless manganese zone. This latter, on drawing air through the column, turns dark brown. Only  $\text{Al}^{+++}$  cannot be detected because its colourless zone is situated between uranyl and zinc and cannot be differentiated from the colourless zinc zone.

**Microchemical Chromatography.**—The cumulative property of the adsorption column which gathers ions present in high dilution in large liquid volumes in a narrow zone makes the method very suitable for the microchemical detection of elements. In the first experiments<sup>1</sup> iron and copper could be detected in amounts down to 1  $\gamma$ . Schwab and Ghosh<sup>2</sup> by using alumina micro columns of 1–2 mm. diam. and some specific microchemical reagents, were able to raise the sensitivity of the method to that

<sup>2</sup> Schwab and Ghosh, *Angew. Chem.*, 1940, **53**, 39.

of Feigl's <sup>10</sup> well-known spot-test method. The advantage of the chromatographic method is that these limits of sensitivity can be attained even at the highest dilutions. Table I (opposite) summarizes the results. Generally, the sensitivity runs parallel with that of the spot test, because it depends on the same factors, viz., colour and solubility of the precipitate. Elements having a low place in the adsorption series, such as Ag<sup>+</sup> or Tl<sup>+</sup>, show a relatively lower sensitivity because at high dilution the adsorption of these elements may be, in part, reversible. In mixtures, forming double rings, the sensitivity is less: in an equivalent mixture, Fe and Cu can be detected by means of Fe(CN)<sub>6</sub><sup>3-</sup> down to 0.1 γ, and in a mixture Fe/10Cu to 0.5 γ Fe and 6 γ Cu.

**Separation of the Platinum Group.**—The same authors <sup>11</sup> in a recent paper applied the alumina chromatography to the separation of the metals of the platinum group. Here, considerable difficulties are encountered, not specific to the method but to the chemistry of these elements. Formation of complex cations and anions, hydrolysis and hydrate isomerism and the ageing of the solutions due to these reactions affect the reproducibility of the results. Osmium has not been included because it is easily removed by the tetroxide distillation. No general developer is available nor is, in fact, needed, as the colours of the different zones are sufficiently characteristic: Ir blue-green, Ru green, Pt faint yellow, Pd brown, Rh orange. The adsorption series is as above from top to bottom, provided that an aged IrCl<sub>3</sub> solution and a fresh RuCl<sub>3</sub> solution are used. The rhodium solution must not be freshly prepared nor older than a few weeks, otherwise the series is seriously disturbed.

The above colours of the single zones are not changed by the presence of more than one platinum metal and thus are reliable means of detection. This has been proved by isolating the free metals from the different zones of the chromatograms and identifying them by measuring their lattice constants in precision X-ray work.

The platinum metals are easily separated from lead, copper, zinc and nickel, but not from iron, because of their higher adsorbability. With iron present, the platinum metals form very complicated chromatograms, due probably to the formation of mixed complexes.

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<sup>10</sup> Feigl, *Qualitative Analyse mit Hilfe von Tropfen-Reaktionen* (Leipzig, 1938).

<sup>11</sup> Schwab and Ghosh, *Z. anorg. Chem.*, 1949 (O. Hahn vol.), **258**, 323.

## THE MECHANISM OF INORGANIC CHROMATOGRAPHIC ADSORPTION ON ALUMINA

BY LUIGI SACCONI

*Received 8th August, 1949*

Adsorption of inorganic salts on activated alumina is considered as a hydrolytic adsorption associated with Al<sup>3+</sup> ion-H<sup>+</sup> ion and Na<sup>+</sup> ion-H<sup>+</sup> ion exchange processes. Alumina, in this way, exhibits a surface buffering action. The adsorption affinity is proportional to the polarizing power exerted by the ions as well as to the polarizability of the groups co-ordinated around the ions. The co-ordinated water molecules of aquo-ions play a peculiar role in connection with surface hydrolysis and with the high tendency of alumina to hydrate itself. The heat of hydration of alumina is a measure of its adsorptive capacity.

Several hypotheses concerned with inorganic chromatography using alumina ascribe the adsorption of electrolytes to the following: (a)  $\text{Na}^+$  ion-cation exchange associated with the presence of sodium aluminate as an impurity<sup>1</sup>; (b) precipitation of basic salts caused by the alkaline reaction of technical alumina<sup>1</sup>; (c) precipitation of basic carbonates due to alkali carbonates and bicarbonates present in the alumina<sup>2</sup>; (d)  $\text{Al}^{+++}$  ion-cation exchange.<sup>1</sup> (This latter hypothesis has now been rejected by the author himself. Thus, the fact that the position of Al in the chromatographic series has been located between Pb and Cu means that the adsorption of ions following the  $\text{Al}^{+++}$  ion cannot be explained.) (e) Amphoteric adsorption by alumina which engages in both  $\text{H}^+$  ion-cation and  $\text{OH}^-$ -anion exchange; (f) preferential adsorption of ions which then bind the ion of opposite charge by equivalent secondary adsorption; (g) molecular adsorption.<sup>3</sup>

It has been shown<sup>4</sup> that Na-free alumina, such as that obtained from amalgamated aluminium, activated by heating for 10 hr. at  $650^\circ\text{C}$ , adsorbs cations, generally in the form of the basic salts, though pure alumina is not basic. Thus, solutions of  $\text{Hg}(\text{NO}_3)_2$  and  $\text{TiCl}_3$ , poured down a column of pure alumina, produce coloured bands (yellow-ochre and brown) of the respective basic salts. It must be emphasized that the filtrate obtained by prewashing the column of technical alumina with water precipitates yellow basic salts from  $\text{Hg}(\text{NO}_3)_2$  solutions, but that the wash water coming from pure  $\text{Al}_2\text{O}_3$  has no effect. We can conclude therefore that the adsorption mechanisms (a), (b) and (c) are not essential.

**Surface Buffering Action of Alumina.**—Interesting information about the nature of the adsorption process has already been obtained<sup>5</sup>:

(1) Pure activated alumina, added to solutions of acids and salts, lowers the hydrogen ion concentration. For instance, the addition of  $\text{Al}_2\text{O}_3$  to a 0.1 N HCl solution until a thick suspension is obtained raises the pH above 5.6. Similar additions to 0.2 M  $\text{CuSO}_4$  and 0.2 M  $\text{NiCl}_2$  solutions (pH = 3.45 and 4.22 respectively) raises the pH above 5.8.

(2) Using as a developer aluminon ( $\text{NH}_4$  aurin tricarboxylate), which does not produce a characteristic reaction either with technical or pure alumina, evidence is obtained that in both static and chromatographic adsorption of acid solutions (e.g., 0.1 N HCl),  $\text{Al}^{+++}$  ions are displaced. These are subsequently adsorbed on the column and produce, with aluminon, a scarlet band at the top of the column. During static adsorption the displaced  $\text{Al}^{+++}$  ion can be detected in the solution; on the column adsorption is complete. Even solutions of salts generally employed in inorganic chromatography can displace  $\text{Al}^{+++}$  ions which are then adsorbed at the top of the column. Thus, pouring  $\text{CuSO}_4$  or  $\text{Fe}_2(\text{SO}_4)_3$  solutions through a column of alumina and developing with aluminon, the white ring situated above the bands of Cu (blue) or Fe (brown) becomes scarlet red. The adsorption band of  $\text{Al}^{+++}$  and other ions, however, are partially mixed.

The results of measurements of the width of Al bands obtained on passing  $\text{H}_2\text{SO}_4$  and  $\text{Al}_2(\text{SO}_4)_3$  solution through columns of pure alumina (diam. 3.5 mm.) are reported in Table I.

The lower part (one-third of the length) of the red zone obtained using  $\text{H}_2\text{SO}_4$  is pale scarlet. The colour thus seems to depend on the different degree of basicity of the basic aluminium salts adsorbed on the column.

(3) In the static adsorption of solutions of acids (e.g., HCl) or hydrolyzable salts

<sup>1</sup> Schwab and Jockers, *Angew. Chem.*, 1937, **50**, 546. Schwab and Dattler, *ibid.* 1937, **50**, 691; *ibid.*, 1938, **51**, 709.

<sup>2</sup> Siewert and Jungnickel, *Ber. B.*, 1943, **76**, 210.

<sup>3</sup> Jacobs and Tompkins, *Trans. Faraday Soc.*, 1945, **41**, 388, 395, 400.

<sup>4</sup> Sacconi, *Gazz. chim. ital.*, 1948, **78**, 583.

<sup>5</sup> Sacconi, *Gazz. chim. ital.*, 1949, **79**, 141; *Nature*, 1949, **164**, 70.

\* Technical alumina employed in these experiments was activated by heating for 10 hr. at  $600^\circ\text{C}$ ; pure alumina obtained from amalgamated aluminium was activated by heating for 10 hr. at  $650^\circ\text{C}$ .

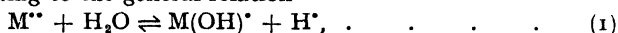
on pure  $\text{Al}_2\text{O}_3$ , the concentration of anions is lowered. On an adsorption column, in which the amount of  $\text{Al}_2\text{O}_3$  is in excess of that of the adsorbate, anion adsorption is complete, i.e., anions cannot be detected in the filtrate coming from the column.

From such experiments the following conclusions are deduced: (a)  $\text{Al}^{+++}$  ions are displaced from alumina by  $\text{H}^+$  ions present in solutions of acid or hydrolyzable salts; (b)  $\text{Al}^{+++}$  ions are subsequently adsorbed, not between the Pb and Cu bands, but together with the most strongly adsorbed ions. This accounts for the absence of  $\text{Al}^{+++}$  ions in solutions coming from the adsorption column. Alumina therefore by lowering the  $\text{H}^+$  ion concentration effects a surface buffering action.

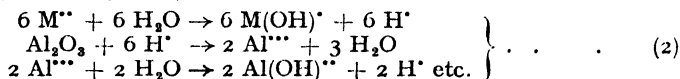
TABLE I

Solution	Vol. (ml.)	Vol. of wash water (ml.)	Vol. of developer (Aluminon soln.) (ml.)	Width of red band (mm.)
0.1 N $\text{H}_2\text{SO}_4$	0.5	2	2	14.5
	0.25	1	1.5	11
0.1 N $\text{Al}_2(\text{SO}_4)_3$	0.5	2	2	11.5
	0.25	1	1.5	7.5

**Hydrolytic Adsorption on Activated Alumina.**—The adsorption of inorganic salts on an alumina column can thus be regarded as a hydrolytic adsorption which takes place according to the general relation



which is displaced towards the right-hand side because of the lowering of hydrogen ion concentration due to  $\text{Al}^{+++}$  ion- $\text{H}^+$  ion exchange. The aluminium salt liberated is then adsorbed as a basic salt:



Since alumina is in excess, the process continues until adsorption is complete. In support of an  $\text{Al}^{+++}$  ion- $\text{H}^+$  ion exchange, attention is drawn to Wagner's conclusions<sup>6</sup> according to which the solution of  $\text{Al}_2\text{O}_3$  in acids is a surface reaction depending not only on the hydrogen ion concentration but also on the concentration of other electrolytes.

In technical alumina  $\text{Na}^+$  ion- $\text{H}^+$  ion exchange also takes place; this accounts for the excess of acid employed in direct neutralization of alumina<sup>7</sup> as well as for the excess adsorption of cations.<sup>1</sup>

The evidence of the different buffering actions of both kinds of alumina can be obtained in the chromatographic adsorption of a  $\text{CuSO}_4 + \text{K}_2\text{Cr}_2\text{O}_7$  solution (orange).<sup>8</sup> A brown band, corresponding to basic copper chromate, is formed on a pure alumina column; a light green band, corresponding to more basic copper chromate, is formed on technical alumina.\*

$\text{CuPtCl}_6$  solution (yellowish green) forms on a pure alumina column two partially mixed zones, the upper yellow, the lower blue-green, corresponding to the basic chloroplatinates of Al and Cu respectively. No effect is observed on washing with water; elution with  $\text{NaOH}$  solution causes the yellow zone to flow down the column ( $\text{Na}_2\text{PtCl}_6$  is not adsorbed) and the green band to turn blue because of the formation of  $\text{Cu}(\text{OH})_2$ . On a column of technical alumina, an upper yellow ring and a blue-green band are likewise formed; on washing with water, the excess of  $\text{Na}_2\text{PtCl}_6$  (yellow), produced by the  $\text{PtCl}_6^{--}$  and  $\text{Na}^+$  ions present in technical alumina, flows down the column.

<sup>6</sup> Wagner, *Z. Elektrochem.*, 1938, **44**, 511.

<sup>7</sup> Schwab, Siewert and Jungnickel, *Z. anorg. Chem.*, 1944, **252**, 321.

\* Basic copper chromates, e.g.,  $7\text{CuO} \cdot \text{CrO}_3 \cdot 5\text{H}_2\text{O}$  (greenish yellow),  $7\text{CuO} \cdot 2\text{CrO}_3 \cdot 5\text{H}_2\text{O}$  (yellow),  $3\text{CuO} \cdot \text{CrO}_3 \cdot 2\text{H}_2\text{O}$  (brown), have been reported. Cf. Gmelin-Kraut *Handbuch der anorganischen Chemie*, Band. V, Abt. 1, 1909, pp. 1188-91.

Further evidence of hydrolytic adsorption is the following: the easily hydrolyzable  $\text{Na}_2\text{PdCl}_4$ ,  $\text{Na}_2\text{RuCl}_4$  and  $\text{Na}_2\text{RhCl}_4$  salts are strongly adsorbed on both pure and technical alumina;  $\text{Na}_2\text{PtCl}_4$ ,  $\text{Na}_2\text{IrCl}_4$  and  $\text{Na}_2\text{OsCl}_4$ , which are stable towards alkali, are not adsorbed. This behaviour provides a method for the complete separation of Pd, Ru, Rh from Pt, Ir, Os.

The decrease of cations adsorption with increasing acidity observed by Jacobs and Tompkins<sup>8</sup> can be explained by the above hypothesis, since an increase in the concentration of hydrogen ion causes the reaction (1) to shift to the left.

The hypothesis (e) that the hydroxyl groups are responsible for adsorption is at variance with the adsorptive capacity exhibited by activated alumina which has been heated at  $600^\circ$ ; this treatment probably causes the bonding of surface hydroxyl groups with elimination of water.<sup>7b</sup> Experimental evidence,<sup>1</sup> furthermore, shows the inability of any cation and anion to replace  $\text{H}^+$  ion and  $\text{OH}^-$  ion respectively. On the contrary, cation- $\text{H}^+$  ion and anion- $\text{OH}^-$  ion exchanges are always observed.

The hypothesis of hydrolytic adsorption appears to be in harmony with Schwab's spectrographic observations,<sup>8</sup> according to which the wavelengths of the bands and the adsorption coefficients of the adsorbed salts agree with those of the corresponding basic salts. This hypothesis is consistent moreover with the experimental evidence of incomplete separation of ions, probably due to co-precipitation phenomena as well as to the fact that the pH values at which the precipitation of several basic salts begins are close to one another.

It must also be remembered that the diffusion coefficient of hydrolyzable salts decreases with the increasing pH of solutions. The diffusion coefficient of  $\text{Al}(\text{NO}_3)_3$ , for instance, becomes zero<sup>9</sup> at  $\text{pH} = 4.5$ . Thus the decrease of the diffusion coefficient of a salt solution which is passing through the interstices in the alumina column causes the corresponding basic salts to be adsorbed.

**Relation Between Adsorbability, Ionic Potential, Diffusion and Dialysis Coefficient of Ions.**—Despite the fact that cations are, in general, not completely separable, and that mixed zones are produced on the column, from my experiments as well as from those already reported by Schwab, the order of cation adsorption on an alumina column is as follows:

Th, Zr,  $\text{Fe}^{\text{III}}$   
 Al, Cr,  $\text{Ti}^{\text{IV}}$ ,  $\text{Hg}^{\text{II}}$ ,  $\text{UO}_2^{\text{II}}$ , Pb, Cu, Ag, Zn,  $\text{Fe}^{\text{II}}$ , Ni,  $\text{Ti}^{\text{II}}$ , Mn.  
 $\text{U}^{\text{IV}}$ ,  $\text{Ce}^{\text{IV}}$ ,  $\text{Ce}^{\text{III}}$

If we consider the degree of hydrolysis  $h^{10}$  of chlorides, nitrates and sulphates of many elements above, we observe that, as a general rule,  $h$  values decrease from Th to Mn (cf. Table II).

TABLE II

Salt	$h$ (%)	pH
Th sulphate .. .. .	46-5	1.8
Th nitrate .. .. .	5.7	
Zr chloride .. .. .	35	
Fe chloride .. .. .	37	
Fe sulphate .. .. .	11.7-22.1	
Cr sulphate (green and violet)	28.7-2.2	1.94-3.2
Cr chloride (green and blue) ..	16.4-1.6	2.2-3.2
Al chloride .. .. .	2.8-4.06	2.76-2.9
Al sulphate .. .. .	1.16-2.01	2.84-2.9
Al nitrate .. .. .		3.17-3.38
Hg chloride .. .. .	0.26	
Pb nitrate .. .. .	0.069-0.084	4.07-4.28
Cu sulphate .. .. .	0.072	4.14
Zn sulphate .. .. .	0.0046-0.0042	5.34-5.28
Cd sulphate .. .. .	0.0016	5.8

<sup>7b</sup> Montoro and De Angelis, *Ricerca sci.*, 1942, **23**, 186.

<sup>8</sup> Schwab and Issidoridis, *Z. physik. Chem.*, 1942, **53**, 1.

<sup>9</sup> Jander and Winkel, *Z. anorg. Chem.*, 1930, **193**, 1; *ibid.*, 1931, **200**, 257.

<sup>10</sup> Landolt-Börnstein, *Physikalisch-Chemische Tabellen*, Hw. II 1165, Eg. I 661, Eg. IIb 1104, Eg. IIIc 2130-33.

Moreover, if we consider the values of ionic potential  $v = z/r$ , dialysis coefficient <sup>11</sup>  $\lambda$  and diffusion coefficient <sup>12</sup>  $D$ , we observe that, in general, the increase of adsorbability runs parallel with increase of  $v$ ,  $1/\lambda$  and  $1/D$ .<sup>13</sup> In addition, more highly hydrated cations <sup>14</sup> are the most highly adsorbed.<sup>15</sup> This is shown in Table III.

TABLE III

Ion	$v = z/r$	$1/\lambda$	$1/D$	$n \text{ H}_2\text{O}$
Ti <sup>IV</sup>	6.25			
Al	5.27	3.29	2.78	57
Cr	4.69	3.20	3.22	49
Zr	4.60			
Fe <sup>III</sup>	4.49	3.37	2.70	59
Ce <sup>IV</sup>	3.92			
U <sup>IV</sup>	3.81			
Th	3.64	3.62	3.45	61
Tl <sup>III</sup>	2.86			
Ce <sup>III</sup>	2.54	2.86	2.78	33
Hg	1.78			
Pb	1.52			
Ag	0.85			
Cu		2.14	2.32	24
Zn	2.41		2.50	
Ni	2.56	2.15	2.38	24
Co	2.44	2.17		24
Fe <sup>II</sup>	2.41	2.18		24
Cd	1.94			
Tl	0.67		0.83	
Mn	2.20	2.20		24

This behaviour is consistent with the recent views on hydrolysis and its correlation with ionic hydration.<sup>16</sup> Thus experiments have shown that the cobaltamines have no water of hydration <sup>16</sup> except that which is held within the co-ordination shell; in this case the co-ordination water is stoichiometrically determined.

On technical alumina cobaltamine cations are adsorbed in the following order; the ionic potential, calculated on the Stokes radii,<sup>17</sup> is also reported. It must be noted that the separation of cobaltamines, when it takes place, is complete. Developers have not been employed except for the  $[\text{Co}(\text{H}_2\text{O})_6]^{2+}$  ion.

- (1)  $[\text{Co}(\text{NH}_3)_3(\text{H}_2\text{O})_3]^{3+}$ ;  $[\text{Co}(\text{NH}_3)_4(\text{H}_2\text{O})_2]^{2+}$ ,  $v = 1.09$ ;  $[\text{Co}(\text{NH}_3)_5(\text{H}_2\text{O})_2\text{Cl}]^{2+}$
- (2)  $[\text{Co}(\text{H}_2\text{O})_6]^{2+}$ ,  $v = 0.58$
- (3)  $[\text{Co}(\text{NH}_3)_6]^{3+}$ ,  $v = 1.02$
- (4)  $[\text{Co}(\text{NH}_3)_5\text{Cl}]^{2+}$ ,  $v = 0.77$ ;  $[\text{Co}(\text{NH}_3)_4\text{CO}_3]^{+}$ ,  $v = 0.37$
- (5)  $[\text{Co}(\text{NH}_3)_4\text{Cl}_2]^{+}$ ;  $[\text{Co}(\text{NH}_3)_6\text{NO}_2]^{2+}$ ,  $v = 0.72$

It is evident that cations with co-ordination water are the most highly adsorbed even when they possess a lower ionic potential. It is therefore concluded that the water of hydration plays an important part in adsorption on alumina.

**Relation Between Hydration and Adsorption.**—It must be emphasized that in inorganic chromatography, where the solvent (water) is highly polar, there are three interacting substances: alumina, ions and water. It is well known that the co-ordination water of aquo-ions (because of the action of the

<sup>11</sup> Brintzinger, Ratanarat and Osswald, *Z. anorg. Chem.*, 1935, **223**, 101.

<sup>12</sup> Spandau, *Z. physik. Chem.*, 1943, **192**, 211.

<sup>13</sup> Sacconi, *Gazz. chim. ital.*, 1949, **79**, 152.

<sup>14</sup> Brintzinger and Ratanarat, *Z. anorg. Chem.*, 1934, **222**, 113.

<sup>15</sup> Emeléus and Anderson, *Modern Aspects of Inorganic Chemistry* (Routledge, London, 1947), p. 148.

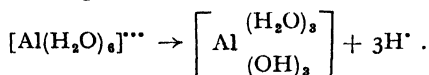
<sup>16</sup> Sutra, *J. chim. phys.*, 1946, **43**, 205. Brintzinger, *Z. anorg. Chem.*, 1935, **225**, 221. Brintzinger, *Z. Elektrochem.*, 1945, **51**, 10.

<sup>17</sup> Sutra, *J. chim. phys.*, 1946, **43**, 193, 194.

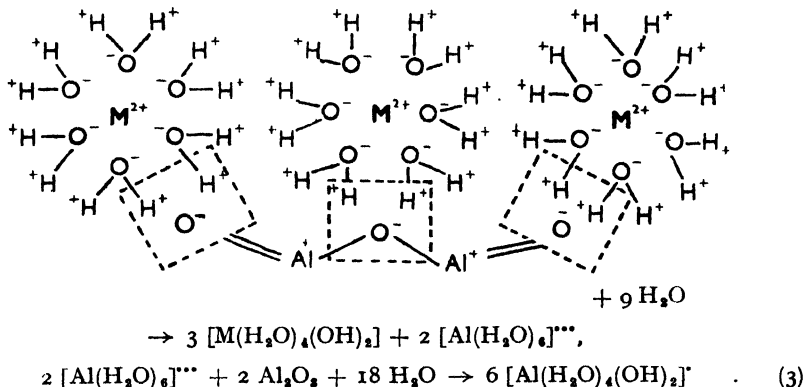
electrostatic field of cation) is polarized and the molecules are oriented with the H atoms directed outwards. Analogous polarization but with the opposite orientation is assumed by water when attracted by alumina which is itself highly polar. In this case the H atoms of water are directed toward the negative ends (oxygen) of the molecules of the adsorbent. Consequently, the polarized water molecules of the aquo-ions will exhibit a tendency to be adsorbed more firmly on alumina than the bulk water in which the molecules are neither polarized nor oriented. Therefore alumina will exhibit a greater adsorption potential towards the water molecules of aquo-ions and in this way adsorb the ions attached to their own water of co-ordination.

Evidently, the greater the potential of ion, the greater is the polarizing action on molecules of water of co-ordination and the greater is the adsorptive capacity of alumina towards the ion itself. If molecules or ions such as  $\text{H}_2\text{O}$ ,  $\text{NH}_3$ ,  $\text{CO}_3^{2-}$ ,  $\text{Cl}^-$ , etc., are contained in the co-ordination shell, the adsorption affinity will depend upon the size, dipole moment and polarizability of the co-ordinate groups. In this manner a reason is given for the fact that the aquo-cobaltamine cations are more strongly adsorbed than cobaltamine cations (O-H distance = 0.955 Å; N-H distance = 1.08 Å;  $\mu_{\text{H}_2\text{O}} = 1.8$ ;  $\mu_{\text{NH}_3} = 1.48$ ).

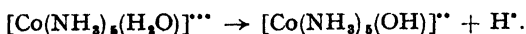
**The Mechanism of Surface Hydrolysis.**—It is clear that compounds containing co-ordinated water are essentially acids in the sense defined by Brönsted,<sup>18</sup> i.e., the polarizing action of the cation creates a tendency to split off hydrogen ions, according to the scheme:



In this way the phenomenon of hydrolysis is explained.<sup>19</sup> In the case of aquo-ions adsorbed on alumina, it is evident that, because of the additional polarization induced by alumina in the co-ordinated water molecule, the production of hydrogen ions and the subsequent salt hydrolysis takes place more easily. The hydrogen ions thus liberated will displace  $\text{Al}^{3+}$  ions from alumina according to a scheme of the type:



The same must be true for aquo-cobaltamine complexes. These, unlike the cobaltamine complexes, exhibit a well-defined tendency to produce  $\text{H}^+$  ions<sup>18, 20</sup> by the reaction:



This provides an additional reason why aquo-cobaltamine cations are more strongly adsorbed than cobaltamine cations.

<sup>18</sup> Cf. Emeléus and Anderson, *loc. cit.*, pp. 147, 230.

<sup>19</sup> Rice, *Electronic Structure and Chemical Binding* (McGraw Hill, New York, 1940), pp. 422, 423.

<sup>20</sup> Pauli and Valkó, *Elektrochemie der Kolloide* (Springer, Wien, 1929), pp. 46, 51.

In this connection attention is directed to Kolthoff's conclusions<sup>21</sup> concerning the adsorption of polar molecules on polar surfaces, according to which the dissociation of the adsorbate takes place when the adsorption energy is greater than the dissociation energy. In the adsorption of aquo-ions on alumina we conclude that, on account of the polarization produced by the electrostatic field of the cations on the co-ordinated water molecules, the adsorption energy necessary to dissociate the latter is lowered and, therefore, the dissociation is facilitated.

Finally it must be noted that, since the adsorption band of any ion is always a mixed zone of both cation and  $Al^{+++}$  ion, measurement of the actual width of the adsorption zone of a single ion is impossible.

**The Heat of Hydration of Activated Alumina.**—Confirmation of the view that inorganic chromatographic adsorption is mainly based on the preferential adsorption by alumina of the polarized water molecules of aquo-ions is provided by the values of the heats of hydration of both technical and pure alumina.<sup>18</sup>

Technical alumina, which is the more active form, has a higher heat of hydration, i.e., a greater tendency to hydrate itself.

The experimental values of the heats of hydration  $Q$  are :

Technical alumina :  $Q = 8.44$  cal./g.

Pure alumina :  $Q = 3.85$  cal./g.

Since the particle size of pure alumina was about 1/10th that of the technical material, the specific heat of hydration of the latter, referred to unit surface, is 20 times that of pure alumina.\*

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<sup>21</sup> Kolthoff, *J. Physic. Chem.*, 1936, **40**, 1027.

## INORGANIC CHROMATOGRAPHY ON CELLULOSE

### Part III.\* The Use of Columns of Cellulose for the Separation and Determination of Metals

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*Received 9th August, 1949*

A process is described for the separation of inorganic materials on columns of cellulose adsorbent with the aid of organic solvents. A method of preparation of a suitable cellulose pulp is given, together with a general description of the practical details of the process. The scope and applicability of the method are illustrated by a variety of separations which have been achieved and some of the factors influencing and producing the separations are considered.

The separation of a number of groups of inorganic radicals on strips of filter paper have been described<sup>1-7</sup> and reference has been made to the use

<sup>1</sup> Arden, Burstall, Davies, Lewis and Linstead, *Nature*, 1948, **162**, 691.

<sup>2</sup> Burstall, Davies, Linstead and Wells, *ibid.*, 1949, **163**, 64.

<sup>3</sup> Arden, Burstall and Linstead, *Chem. Soc. Symposium* (Chemistry of the Heavy Elements), March, 1949.

<sup>4</sup> Lederer, *Nature*, 1948, **162**, 776.

<sup>5</sup> Lederer, *ibid.*, 1949, **163**, 598.

<sup>6</sup> Pollard, McOmie and Elbeih, *ibid.*, 1949, **163**, 292.

<sup>7</sup> Lacourt, Sommereyans, Degeyndt, Baruh and Gillard, *ibid.*, 1949, **163**, 999.

\* A New Method for the Detection and Determination of Uranium by T. V. Arden, F. H. Burstall and R. P. Linstead (in press) should be taken as Part I of this series of papers, Part II, The Separation and Detection of Metals and Acid Radicals on Strips of Absorbent Paper by F. H. Burstall, G. R. Davies, R. P. Linstead and R. A. Wells (in press).



of columns of cellulose for separating larger amounts of material.<sup>3 6</sup> This paper is intended to give a general account of the technique of separating inorganic salts, using columns of cellulose in conjunction with organic solvents, together with an outline of some separations already achieved. Detailed accounts of the individual separations mentioned will be published elsewhere.

The maximum amount of material involved in separations on paper strips is of the order of 1 mg.; larger quantities exceed the capacity of the cellulose and clear-cut separations are not achieved. It is possible, however, to deal with very much larger amounts of material by using columns of cellulose pulp and separations employing up to 250 g. of material have been successfully carried out.

### Experimental

**General Technique.**—A column of cellulose pulp is prepared in a glass tube containing the organic solvent to be used for the separation. An aqueous (usually dilute acid) solution of the mixture to be separated is prepared and transferred to the top of the column. The solution is either poured directly on to the top of the prepared column or is first absorbed on a wad of cellulose, which is then transferred to the top of the tube, broken up with a glass rod and then packed down to form a continuous portion of the column. More regular and concentrated bands are obtained by the latter method. Solvent is then passed down the column and the eluent collected. The material being separated is dissolved, moves down the column and finally emerges in the eluent. Recovery of the extracted material from the solvent eluent is made in one of several ways: (a) washing with dilute acid, (b) addition of water and evaporation of the solvent and (c) precipitation in the organic solvent solution followed by filtration.

**Preparation of Cellulose.**—Various types of cellulose materials including wood pulp, cotton linters and filter paper have been investigated for suitability in pulp preparation. In all cases degradation of the cellulose to a suitable form of pulp is achieved by boiling with an aqueous solution containing 5 ml.  $\text{HNO}_3$  ( $\rho = 1.42$ )/100 ml. The time of boiling required varies from 2 to 20 min. according to the nature of the raw material. The type of pulp used is suited to the separation to be carried out; for a number of routine estimations pulp prepared from Whatmans No. 1 Waste Paper Clippings is satisfactory, but when a higher degree of purity is required Whatmans Ashless Tablets are used. On boiling with water these tablets break down to a pulp suitable for many separations, but a more finely divided and retentive pulp is obtained by boiling with 5 % v/v nitric acid for 2 min.; excess acid is then removed by washing with water, alcohol and finally ether. The boiling with dilute nitric acid breaks down the cellulose fibres and increases the specific surface of the cellulose. The number of active end-groups in the cellulose is also increased and this improves the adsorptive powers of the pulp. The reducing property of a cellulose pulp is an important contributing factor in the separation of inorganic materials since polyvalent metals tend to give, in their lower valency forms, salts which are less soluble in organic solvents.

**Preparation of the Glass Extraction Tube.**—It is necessary to treat the inside surface of the glass extraction tube to prevent 'wall' effects due to creep of aqueous solution from the wad of cellulose containing the sample, down the wall of the absorption column. This wall effect has been overcome by the use of dichlorodimethyl silane  $(\text{CH}_3)_2\text{SiCl}_2$ , which confers very strong water-repellent properties to the glass surface. A small amount of the organic silicon derivative is shaken in the tube until the whole surface has been treated, the excess removed and the tube washed with alcohol. In the absence of fluoride the silicone-treated tube retains its water-repellent properties for a large number of separations.

### Results

In the separation of inorganic materials by the column method a number of unusual chromatographic conditions are experienced. For this reason although

separations carried out by the paper strip technique provide a useful guide as to the best solvent to use in a column separation, the conditions of water and acid concentration are not usually the same for both types of separation. The column process appears normally much more sensitive to small changes in acidity and water content of the solvent than does the strip technique. The following examples will give some account of the separations to which the technique has been successfully applied and on which work is still proceeding.

A mixture of methyl propyl ketone, water, HCl and acetone has been used in the separation and estimation of Ni, Co and Cu in samples of steel and minerals. A solution of the sample (0.2 g.) is prepared in dilute hydrochloric acid (2-5 ml.) and transferred to the top of a cellulose column packed in methyl propyl ketone. The cellulose pulp used is prepared by nitric acid treatment of Whatmans Ashless

TABLE I  
ESTIMATION OF CU, NI AND CO IN VARIOUS SAMPLES

Material	Results by column process %	Mean of column results %	Results by normal analysis %
Nickel-chrome steel	Cobalt 10.00	10.03	10.02
	10.10		
	10.00		
	Nickel 12.85	12.78	12.90
	12.75		
	12.75		
Tungsten steel	Cobalt 4.36	4.36	4.35
	4.40		
	4.31		
	Nickel 0.49	0.46	0.43
	0.44		
Iron pyrites	Copper 2.65	2.68	2.69
	2.71		
	2.68		
	Cobalt 0.13	0.12	0.10
	0.12		
	0.11		
Copper nickel speiss	Copper 17.16	17.11	17.04
	17.02		
	17.16		
	Nickel 22.86	22.91	22.80
	23.00		
	22.86		

Tablets. The chlorides of Fe, Cu and Co are extracted from nickel using methyl propyl ketone containing 1 % v/v HCl ( $\rho = 1.18$ ) as solvent, but clear separation of Fe, Cu and Co is not effected. If the acid concentration, however, is kept low and the water content of the solvent is increased, separation of Co, Cu and Fe is achieved. Thus, using a solvent consisting of 100 parts of methyl propyl ketone, 30 parts of acetone, 4 parts of water and 1 part of HCl ( $\rho = 1.18$ ), iron and copper are extracted from cobalt and nickel. The iron is first removed as a yellow band followed by an amber band of copper, while cobalt remains with the nickel. After the copper band has moved completely out of the column, the solvent is changed and the extraction continued with methyl propyl ketone containing 2 % v/v HCl ( $\rho = 1.18$ ); this solvent extracts cobalt from nickel. The copper is extracted from the requisite eluent fraction by several washings with 2 N HCl. The combined washings are evaporated to dryness, fumed with

HNO<sub>3</sub> to remove organic matter, then evaporated twice with HCl to remove nitrate. The copper is estimated colorimetrically with rubeanic acid on a Spekker absorptiometer. The cobalt fraction is diluted with acetone and the cobalt estimated by means of the colour developed with ammonium thiocyanate. The nickel is extracted from the cellulose column by washing with dilute HCl. The solution is evaporated to dryness fumed with HNO<sub>3</sub> and then evaporated twice with HCl. The nickel is then determined with dimethylglyoxime. Some typical results obtained by this process are shown in Table I.

Mercury as mercuric chloride is extracted quantitatively from copper, bismuth, lead and cadmium using pure dry methyl acetate as solvent. If a normal cellulose column is employed the diffuse rear boundary of the mercury band and the forward boundary of the bismuth band overlap, with consequent contamination of the eluent by traces of bismuth. This effect has been overcome by including in the column a layer of pulp impregnated with sodium phosphate solution. The phosphate does not effect the extraction of mercury but successfully retains the bismuth. By this method 0.2 g. quantities of mercuric chloride have been separated from an equal weight of each of the chlorides of lead, copper, cadmium and bismuth. The mercury is recovered by passing H<sub>2</sub>S gas through the solvent eluent and filtering off the precipitated sulphide.

Other separations which have been carried out in chloride solution include the separation of gold from the platinum metals and the separation of the individual platinum metals. Gold is extracted quantitatively from gram quantities of the platinum metals using ethyl ether containing aqueous HCl and some success has been gained in the preparative separation of the platinum metals using acetone or methyl ethyl ketone containing HCl ( $\rho = 1.18$ ).

A number of separations have also been carried out in nitrate solution. In consequence of the unusually high solubility of uranyl nitrate in organic solvents the extraction of uranium from many other elements has been carried out very easily.

Using ethyl ether containing 12½ % w/v HNO<sub>3</sub> promising results have been achieved in the separation of hafnium and zirconium. The separation is not yet quantitative but has been the means of obtaining samples of zirconium free from hafnium.

### Discussion

The wide applicability of the method has been indicated by the variety of the examples of separation given. It is obvious that there are many factors which can operate to bring about these separations. There are, however, three main factors which contribute to the mechanism of separation to a varying degree with the individual metals.

(a) SELECTIVE EXTRACTION OF THE SALTS BY THE SOLVENT.—This effect plays an important part at the top of the column, where the mixture of salts is situated and is approximately proportional to the relative solubilities of the salts in the organic medium. It does not follow, however, that the order in which the salts reach the bottom of the column is proportional to these solubilities.

(b) PARTITION.—Partition of the inorganic substances occurs between the organic solvent and the water present in the cellulose. The water content of the pulp varies with the water content of the organic solvent and when inorganic salts are added as an aqueous solution the water content of the cellulose at the top of the column is abnormally high (at least at the beginning of the extraction). Changes in the concentration and composition of the salts in the solvent phase, as it moves downwards, lead to variations in partition due to alterations in the salting-out influence of the various salts on each other.

(c) ADSORPTION.—Metals ions are adsorbed to different extents by the cellulose. This factor is affected by activation of the cellulose, for example, by treatment with nitric acid. It is altered by variations in the acid content of the solvent.

In conclusion it is seen that this method of effecting separations opens up a wide field in the analysis (qualitative and quantitative), preparation and even production of inorganic materials.

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## THE APPLICATION TO QUALITATIVE ANALYSIS OF THE SEPARATION OF INORGANIC METALLIC COMPOUNDS ON FILTER PAPER BY PARTITION CHROMATOGRAPHY

BY I. I. M. ELBEIH, J. F. W. McOMIE AND F. H. POLLARD

*Received 12th August, 1949*

An investigation into the separation of inorganic metallic compounds on filter paper by (a) aqueous *n*-butanol containing a complexing reagent and (b) aqueous collidine, and its general application to the separation of metallic compounds by a two-way process, is described. The detection of the position of the compounds on the filter paper is achieved by the use of ultra-violet fluorescence.

A special application of the separation and detection of arsenic from a large number of other metallic compounds is also described.

The use of filter paper for separating metallic derivatives in microchemical methods of qualitative analysis has long been practised. Feigl<sup>1</sup> has discussed these methods, and has pointed out that "the separating action of filter paper, due to capillary phenomena alone, is, as a rule, not sufficient to effect an isolation of the inorganic compounds of a solution into distinct zones." Even when this is possible the compounds are not present alone and in a pure state. The advantage of the new method of paper chromatography is that many inorganic compounds can be separated into quite distinct zones, in most cases several inches apart.

The separation of inorganic compounds on filter paper by the method of partition chromatography has so far developed along two main lines using (a) solvents containing reagents capable of forming complexes with inorganic ions, such as benzoyl acetone, and (b) special solvents, the usefulness of which may depend partly on the formation of complexes by reaction of the solvent with the metal ions under examination.

Linstead and his co-workers,<sup>2</sup> as well as Lederer,<sup>3</sup> have by the use of partition chromatography achieved a large number of important separations, but have tended to concentrate on special separations rather than on general methods of separating ions. Our procedure has obvious advantages in the general field of qualitative analysis. The technique which we have

<sup>1</sup> Feigl, *Specific and Special Reactions* (Elsevier, 1940), p. 162.

<sup>2</sup> Linstead *et al.*, *Nature*, 1948, **162**, 691; *ibid.*, 1949, **163**, 64; *ibid.*, 1949, **163**, 508.

<sup>3</sup> Lederer, *Nature*, 1948, **162**, 776.

developed makes use of both types of solvents, attention being focused in the first case on *n*-butanol containing one of the several well-known reagents which are known to form complex ions with the metals, and secondly on collidine as a special solvent of type (b).

Our general investigations have also led us to achieve the separation of several metals by a two-stage separation process, using first a solvent of type (b), and then one of type (a) moving at right-angles to the first solvent.

Under special conditions, the separation of only one inorganic compound from a large number of others has particular importance, and Linstead<sup>4</sup> has shown that uranium compounds can be separated in this manner, while we have adapted our technique to separate arsenic from a large number of other elements.

It is very convenient to have some general reagent which can be used to detect the position of the ions after separation is complete. We have found that materials which form with metallic ions compounds which fluoresce or suppress fluorescence in ultra-violet light are of great value. After spraying the paper with the reagent and then exposing it to the radiation from a mercury vapour lamp, the position of the ion, invisible in daylight, is revealed. A number of suitable reagents have been investigated and are described in this paper.

### Experimental

**Apparatus.**—The general arrangement of the apparatus is similar to that already in use for organic chromatography, and consists of a glass trough supported in a glass tank, 10 in. × 13 in. × 16 in., the top of which is ground level and covered with a heavy ground glass sheet to ensure that during an experiment the tank is air-tight. In the trough is put the organic solvent layer and at the bottom is a Petrie dish containing the aqueous layer. The tank is placed in an insulated cupboard.

**Preparation of Solvents.**—Two solvent mixtures have been used throughout.

(a) **BUTANOL MIXTURE.**—*n*-Butanol (100 ml.) is first shaken with the required quantity of complexing reagent, until the latter is dissolved. The solution is then thoroughly shaken with dilute nitric acid (100 ml.). The normality of the nitric acid is varied according to the complexing agent in use. After standing overnight to attain equilibrium, the aqueous layer is run off and put in the Petrie dish, while the upper layer is placed in the trough.

(b) **COLLIDINE MIXTURE.**—2 : 4 : 6-Collidine (100 ml.) is shaken with 0.4 N nitric acid (100 ml.), the heat generated by the mixing causing the temperature to rise to about 30° C. When the mixture cools on standing to 25° C, the layers are separated as already described.

**Preparation of the Metal-ion Solutions.**—0.1 N solutions of the nitrates of Ag<sup>+</sup>, Hg<sup>+</sup>, Pb<sup>2+</sup>, Hg<sup>2+</sup>, Bi<sup>3+</sup>, Cu<sup>2+</sup>, Cd<sup>2+</sup>, Al<sup>3+</sup>, Fe<sup>3+</sup>, Zn<sup>2+</sup>, Mn<sup>2+</sup>, Co<sup>2+</sup> and Ni<sup>2+</sup> were prepared, containing sufficient nitric acid to prevent hydrolysis. 0.2 N solutions of the nitrates of Ca<sup>2+</sup>, Sr<sup>2+</sup>, Ba<sup>2+</sup>, Mg<sup>2+</sup>, K<sup>+</sup> and Na<sup>+</sup> were prepared and made slightly acid with nitric acid. 0.2 N solutions of the chlorides of Sn<sup>2+</sup>, Sn<sup>4+</sup> and Cr<sup>3+</sup> were acidified with sufficient hydrochloric acid to prevent hydrolysis. A 0.1 N solution of arsenious oxide in water was slightly acidified with nitric acid. A 0.1 N solution of antimony tartrate was prepared and rendered faintly acid with tartaric acid.

**Procedure.**—Drops of solution to give a spot of about 1 cm. diam. are placed at distances of about 4 cm. apart on a pencilled line 8 cm. from the top edge of the sheet (27 cm. × 40 cm.) of Whatman No. 1 filter paper. Two similar sheets are placed one on each side of the trough, with the top edge dipping into the liquid. The tank is closed by the ground glass plate, placed in the insulated cupboard, and left until the liquid front is about 5 cm. from the

<sup>4</sup> Arden, Burstall and Linstead, *The Chemistry of the Heavy Elements* (Chem. Soc. Symposium, 1949).

lower edge of the paper. This takes about 20 hr. for the butanol mixtures and 35 hr. for the collidine. If the vessel is quite air-tight, and not exposed to varying temperatures, the liquid front remains horizontal.

The sheets of paper are removed from the tank, dried in a stream of hot air, and then sprayed with a special reagent to reveal as many ions as possible. As the unknown can be placed on the same sheet of paper, there is no need to use a specific reagent for each ion, since the unknown can always be compared with the known ions which have travelled down the paper simultaneously. This procedure also avoids the necessity of knowing exact  $R_f$  values. To confirm the identity of the unknown, it is always possible to put an extra spot of the unknown at the side of the paper, a strip of which can be cut off and tested with specific reagents. The position of the unknown will, of course, be obtained from the main strip.

**Detection and Identification of the Ions.**—A spray of ammoniacal aqueous hydrogen sulphide solution serves to reveal the presence of  $\text{Ag}^+$ ,  $\text{Hg}^+$ ,  $\text{Pb}^{2+}$ ,  $\text{Hg}^{2+}$ ,  $\text{Bi}^{3+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Cd}^{2+}$ ,  $\text{As}^{3+}$ ,  $\text{Sb}^{3+}$ ,  $\text{Fe}^{3+}$ ,  $\text{Co}^{2+}$  and  $\text{Ni}^{2+}$  as coloured sulphides.

As indicated in an earlier publication,<sup>5</sup> however, a much more general method is to use organic reagents which, on exposure of the chromatogram to ultra-violet light, reveal the position of the ions as dark or fluorescent spots. The most satisfactory spray for this purpose is a solution of 5 g. 8-hydroxyquinoline and 1 g. kojic acid in 1 l. of 60 % alcohol. After spraying, the paper is exposed to ammonia vapour to make it alkaline. Many other reagents have been tested for this purpose, and some of the more promising results are recorded in Table I.

## Results

### (a) Separations using a Solvent Containing a Complexing Reagent.

Experiments were carried out with butanol mixtures prepared with butanol and dilute nitric acid of different normalities, both with and without complexing reagents. In Fig. 1 the  $R_f$  values for most of the common ions are plotted as a function of the position of the ion on the original chromatogram, using mixtures prepared with N, 2 N, and 3 N nitric acid without a complexing reagent. It is clear that the relative separation is the same in most cases, but that the ions travel further in the more highly acid medium.  $\text{Hg}^+$ ,  $\text{Hg}^{2+}$  and  $\text{Sb}^{3+}$  show pronounced "tailing" under these conditions, and so their positions have not been included.

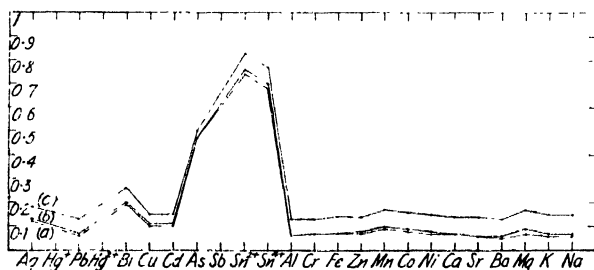


FIG. 1.—Separations with *n*-butanol mixture, without complexing reagent, and prepared with (a) 1 N  $\text{HNO}_3$ ; (b) 2 N  $\text{HNO}_3$ ; (c) 3 N  $\text{HNO}_3$ .

It was found that much better separations could be achieved in some cases by the addition to the liquid medium of an organic reagent, capable of forming complexes. Many such reagents were tried under different conditions of acidity, and among the most successful of those yet tried were benzoyl acetone and acetyl acetone, while dibenzoyl methane, acetoacetic ester and dimethyl glyoxime were not, in general, so good. A typical example of the separations achieved is shown in Fig. 2 for benzoyl acetone, which up to the present has given the best separations and most symmetrical spots.

TABLE I  
ORGANIC REAGENTS FOR DETECTING IONS BY ULTRA-VIOLET FLUORESCENCE

Fluorescent Substance	Bright Spots		Dark Spots		Invisible Spots
	++ +	+ +	+	--	
<i>o</i> -Aminobenzoic Acid ..	—	—	Sb	Ag, Cu, Cd, Mn, Co Hg <sup>+</sup> , Pb, Hg <sup>2+</sup> , Bi, Al, Fe, Zn, Ni	Cr, Ca, Sr, Ba, Mg, K, Na As, Sn <sup>2+</sup> , Sn <sup>4+</sup>
8-Hydroxyquinoline ..	Cd, Al, Zn, Mg	Ag, Sn <sup>2+</sup> , Sn <sup>4+</sup> , Ca	Sr, Ba	Fe Hg <sup>+</sup> , Hg <sup>2+</sup> , Bi, Cu, Sb, Mn, Co, Ni	Pb, Cr, K, Na As
Kojic Acid ..	Ag, Hg <sup>+</sup> , Hg <sup>2+</sup>	Ca	Sr, Ba, Mg	Cu, Fe, Ni Bi, Al, Mn, Co	Pb, Cd, Sb, Sn <sup>2+</sup> , Sn <sup>4+</sup> , Cr, Zn, K, Na As
Naphthionic Acid ..	—	—	—	Hg <sup>2+</sup> , Fe, Mn Ag, Hg <sup>+</sup> , Pb, Bi, Cu, Cd, Al, Cr, Zn, Co, Ni, Ca, Sr, Ba, Mg, K, Na	— As, Sb, Sn <sup>2+</sup> , Sn <sup>4+</sup>
Morin ..	Al, Zn	Pb, Cd, Sn <sup>2+</sup> , Sn <sup>4+</sup> , Mg	—	Cu, Fe Ag, Hg <sup>+</sup> , Hg <sup>2+</sup> , Bi, Cr, Mn, Co, Ni	— As, Sb, Ca, Sr, Ba, K, Na

NOTE.—Valencies have been given only to those metals present in more than one valency state.

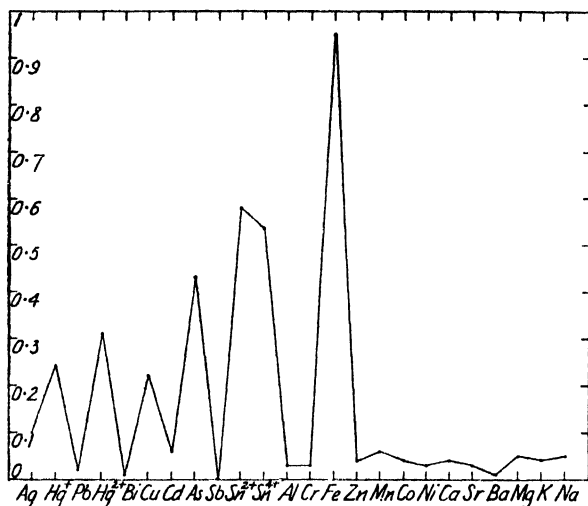


FIG. 2.—Separations with *n*-butanol mixture prepared with 0.1 N nitric acid and 0.5 % benzoyl acetone.

(b) **Separations using a Solvent only.**—The most interesting results with this type of solvent have been obtained with collidine, and the separations are illustrated in Fig. 3. Again it was found that the acidity was important for good results.

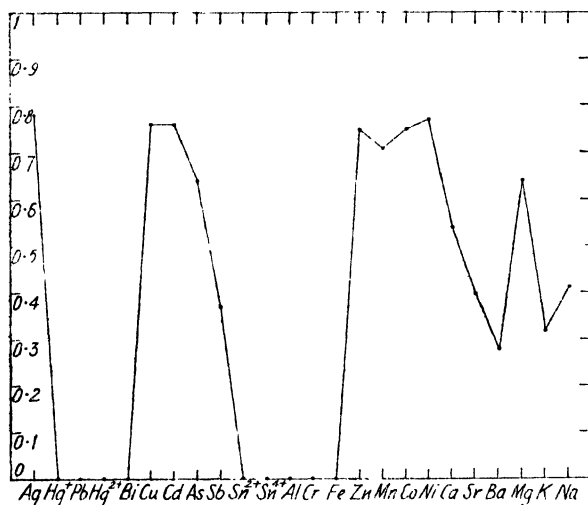


FIG. 3.—Separations with collidine mixture prepared with 0.4 N nitric acid.

(c) **Two-way Separations.**—The possibility of separating cations by using the properties of the two types of solvents has been investigated with a mixture containing  $\text{Al}^{3+}$ ,  $\text{Fe}^{3+}$ ,  $\text{As}^{3+}$ ,  $\text{Cu}^{2+}$  and  $\text{Cd}^{2+}$ . A spot containing all these ions was put at a point X on the filter paper. The collidine solvent was first used, and the compounds of  $\text{As}^{3+}$ ,  $\text{Cd}^{2+}$  and  $\text{Cu}^{2+}$  were thus separated from those of  $\text{Fe}^{3+}$  and  $\text{Al}^{3+}$ . The  $\text{Cu}^{2+}$  and  $\text{Cd}^{2+}$  moved together as predicted from Fig. 3, and the  $\text{As}^{3+}$  did not travel so far.

The filter paper was washed with  $\text{CCl}_4$  and dried, and then the butanol solvent containing benzoyl acetone was used with the paper at right-angles to its original



position. The iron compound now separated from the aluminium; the copper separated from the cadmium and the arsenic moved to a new position; the copper actually moved with a higher  $R_f$  value than predicted from Fig. 2. In this way a clear separation of these five metallic compounds was achieved. Their positions were first detected by fluorescence, and confirmed by hydrogen sulphide, etc. The relative positions of the metallic compounds are illustrated in Fig. 4.

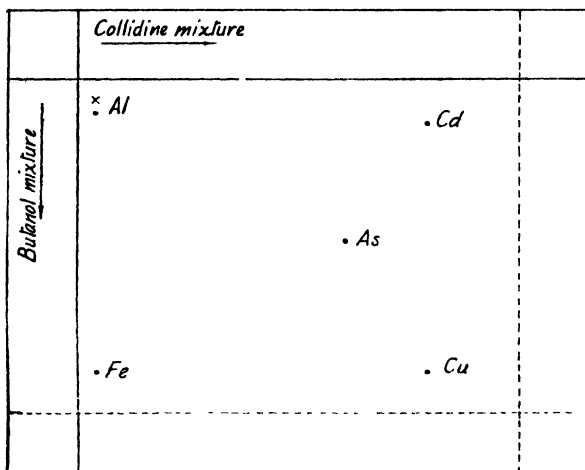


FIG. 4.—Example of two-way separation.

(d) **Separation of one Ion from a Large Number of Others.**—As an example of the application of this type of separation, arsenic was selected because of its obvious importance. The fundamental technique of using butanol-water mixtures was adopted, and the effect of various substances was investigated to try to obtain large  $R_f$  values for arsenic while retaining small  $R_f$  values for other cations present. The best mixture proved to be butanol and water prepared with 1 % ammonium borate, 1 % ammonium tartrate, 0.5 % mannitol. The actual  $R_f$  values for all the common cations using this mixture are given in Fig. 5, from which the exceptional behaviour of arsenic is obvious.

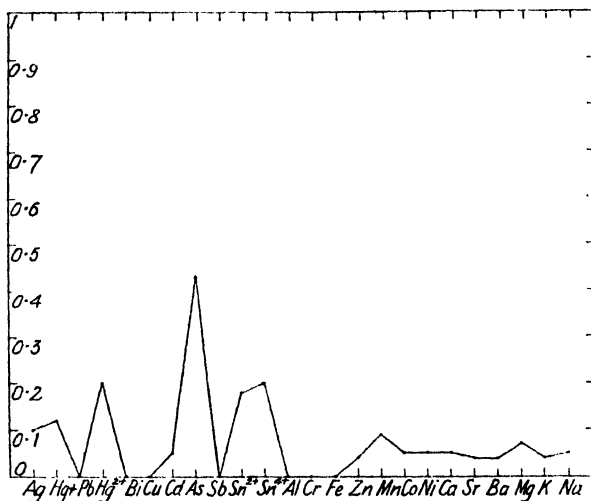


FIG. 5.—Separation of arsenic from other metallic compounds.

A special test for arsenic was developed as follows. The chromatogram was sprayed with a mixture of alcohol containing 1 % conc. nitric acid and 5 % glycerol to make the paper acid, and then, after drying, sprayed with ammoniacal silver nitrate. The arsenic spot is converted, presumably to silver arsenite (yellow), which, on irradiation with ultra-violet light whilst still wet, changes to silver arsenate (brown) and metallic arsenic (black). The spot is then easily visible against the purplish background from the silver nitrate spray. With stationary drops of 0.01 ml. taken by a micrometer syringe from standard solutions of sodium arsenite it was possible to detect 0.1  $\gamma$  of arsenic; on the chromatogram, however, the limit of sensitivity was 0.3  $\gamma$  of arsenic. The test can be made quantitative and used for a colorimetric estimation of the arsenic.

### Discussion

The illustrations, Fig. 2 and 3, show the kind of separations which are possible for two different types of solvents. It is clear that the cations of their derivatives can be divided into two groups, depending on the movement in the two different solvents.

GROUP A. Cations moving with butanol-water-benzoyl acetone mixture.

$\text{Ag}^+$ ,  $\text{Hg}^+$ ,  $\text{Hg}^{2+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Cd}^{2+}$ ,  $\text{As}^{3+}$ ,  $\text{Sn}^{2+}$ ,  $\text{Sn}^{4+}$ ,  $\text{Fe}^{3+}$ .

GROUP B. Cations moving with collidine-water mixture.

$\text{Ag}^+$ ,  $\text{Cu}^{2+}$ ,  $\text{Cd}^{2+}$ ,  $\text{As}^{3+}$ ,  $\text{Sb}^{3+}$ ,  $\text{Zn}^{2+}$ ,  $\text{Mn}^{3+}$ ,  $\text{Co}$ ,  $\text{Ni}$ ,  $\text{Ca}^{2+}$ ,  $\text{Sr}^{2+}$ ,  $\text{Ba}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{K}^+$ ,  $\text{Na}^+$ .

While some inorganic compounds move in both solvents the extent of the movement may vary considerably; thus  $\text{Ag}^+$ ,  $\text{Cu}^{2+}$ ,  $\text{Cd}^{2+}$ ,  $\text{As}^{3+}$  derivatives move much further in collidine than they do in butanol. Also, it should be noted that, while copper and cadmium compounds move together in collidine, there is quite a good separation in the butanol mixture. The separation of sodium, potassium and magnesium by the collidine mixture is particularly interesting.

There is some evidence that formation of complexes is essential for rapid movement, if one compares the results of butanol mixtures with and without complexing reagents as illustrated by Fig. 1 and 2. It is noticed that when no complexing reagent is present, movement of several cations, for example,  $\text{Bi}^{3+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Cd}^{2+}$ ,  $\text{As}^{3+}$ ,  $\text{Sn}^{2+}$ ,  $\text{Sn}^{4+}$ , etc., occurs, and that the movement is greater for the more acidic mixtures. The effect of the increased acidity is probably associated with the prevention of hydrolysis of these particular nitrates, and consequently the nitrates are capable of movement. In the presence of a complexing reagent, and providing the acidity is not too great, that is, for benzoyl acetone not greater than about 0.1 N, the movement of the various ions differs considerably. Under such conditions, one obtains, for example, as has been pointed out already, a separation of copper and cadmium. If, however, the acidity is greater than 0.1 N, for benzoyl acetone the movement of the ions in general resembles that without any complexing reagent being present, and there is no separation of copper and cadmium. Presumably this is due to the instability of the complexes in strongly acid solution, but at the same time it is necessary to have the solution sufficiently acid to prevent undue hydrolysis of some of the original nitrates. For any particular complexing reagent it is found necessary to determine the optimum conditions.

At present there is insufficient evidence for much to be said about the mechanism of these particular chromatographic processes, but it does seem that for their successful operation the formation of complexes is essential. As collidine itself is quite likely to form complexes with the cations, then in this type of solvent complex formation probably plays an important role.

How far true partition between solvents occurs is still uncertain. In our earlier communication<sup>5</sup> we pointed out that for butanol mixtures water alone washes the ions down the paper, while butanol mixture prepared with 10 % acetic acid produces almost no movement. This seemed to indicate that in this case it was essentially a partition process.

The preliminary investigations on two-way separations indicate that our technique is capable of very general application for the separation of the cations common to qualitative analysis, and the special separation of arsenic is being developed for the quantitative estimation of small quantities of arsenic.

Our thanks are due to Dr. J. K. N. Jones for his great interest in this work, and to the Egyptian Education authorities for a grant to one of us (I. I. M. E.).

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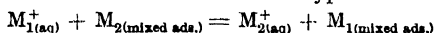
## INORGANIC CAPILLARY ANALYSES

By H. FLOOD

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The paper deals with chromatographic experiments using porous paper impregnated with  $\text{Al}(\text{OH})_3$  as adsorbent. The paper is cut in strips, a small amount of the solution is sucked up at one end, followed by a larger amount of pure solvent. The chromatographic zones are developed by brushing with suitable reagents. The method may be used for the analyses of cation or anion mixtures. An anion chromatogram is formed when a strong acid is sucked into the paper prior to treatment with the solution.

The sequence in which the cations are adsorbed is the same as found by Schwab with few exceptions. The effect of the presence of anions and other factors influencing the length of the molar adsorption zone of the cations are discussed. If the less adsorbable  $\text{M}_2^+$  ion is added to a solution of  $\text{M}_1^+$  ion an increase is observed in the adsorption zone of  $\text{M}_1^+$ . The reason for this is that a mixed zone of  $\text{M}_1$  and  $\text{M}_2$  is found. Approximate values of the exchange constant of reactions of the type :



have been derived. From the values of these constants the possibility of a quantitative separation of cations in mixtures can be predicted. The effects of complex-forming reagents are also estimated when the complex constants are known. This is demonstrated by the chromatograms of solutions to which glycinate has been added. Addition of complex-forming reagents may also be used for a simple quantitative chromatographic titration.

The investigations reported in the present paper were chiefly carried out during 1938 to 1945. The method itself has been described<sup>1</sup> but as a consequence of the wartime conditions most of the results were either partly published in Norwegian (with only a brief summary in German<sup>2 3 4 5 6</sup>) or remained unpublished.

<sup>1</sup> Flood, *Z. anal. Chem.*, 1940, **120**, 237.

<sup>2</sup> Flood, *Tidsskr. kjemi bergvesen*, 1940, **20**, III.

<sup>3</sup> Flood and Smedsaas, *Tidsskr. kjemi bergvesen metallurgi*, 1941, **1**, 150.

<sup>4</sup> Flood and Smedsaas, *Tidsskr. kjemi bergvesen metallurgi*, 1942, **2**, 1.

<sup>5</sup> Flood and Smedsaas, *Tidsskr. kjemi bergvesen metallurgi*, 1942, **2**, 17.

<sup>6</sup> Flood, *Tidsskr. kjemi bergvesen metallurgi*, 1943, **3**, 9.

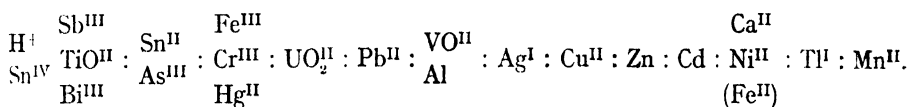
The characteristic feature of the method is that porous paper impregnated with  $\text{Al}(\text{OH})_3$  is used as adsorbent. Some solution, followed by pure water, is sucked up into a strip of the paper. Thick blotting paper is most suitable since it possesses a homogeneous distribution of pores. The impregnation is carried out by dipping the paper in a solution of sodium aluminate (0.1 to 1 mole/l.). The paper is then dried and the aluminium hydroxide formed by dipping it into a saturated solution of  $\text{NaHCO}_3$ . The paper is washed for several days in distilled water, dried over a period of some days and finally cut into strips ( $30 \times 1$  cm. or smaller).

The solution is sucked up in the one end of the strip to a height of 1-3 cm. (For quantitative purposes the amount ( $\approx 0.1$  ml.) is measured with a capillary pipette.) The paper is then treated with water until a height of 10 cm. or more is wetted. Two or three times the length of the adsorption zone is sufficient to give a constant length of zone. During the passage of water the adsorption zone does not leave the lower end of the paper. This indicates that the chief adsorption process is an exchange between cations from the solution and sodium ions from aluminate impurities in the aluminium hydroxide. Finally the chromatographic zones are developed by brushing with suitable reagents.

Some advantages of this method compared with powder columns may be noted. The manner of development causes no disturbance of the primary zones. Several developing reagents can be used in the same chromatogram, after cutting up the strip into 2 or more parts. The method is well suited to semi-micro work (1 mg.) and can also be modified for micro work (1  $\gamma$ ). Most important, however, is that the results can be reproduced since strips from the same sheet normally show nearly the same adsorptive capacity.

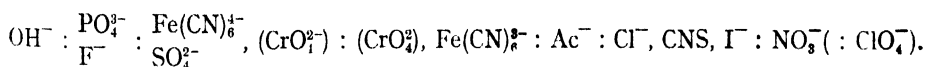
The results are easily reproduced within  $\pm 5\%$  and by careful work to  $\pm 2\%$  may be reached. It must be noted that the height of an adsorption zone is sensitive to depression of the surface tension of the solution. Details, especially concerning the quantitative use of the method, have been given previously.<sup>1</sup>

**1. Sequence of Adsorption.**—The following sequence of adsorption is found for cations:



With few exceptions (Cd-Co, Ag-Cu) this is the same as previously found by Schwab and Jockers<sup>7</sup> on  $\text{Al}_2\text{O}_3$  columns. Ca, Sr and Ba form mixed zones with the transition elements (Cu, Ni, etc.). Mg apparently is adsorbed, but the zone moves slowly upwards with the washing water (precipitation of  $\text{Mg}(\text{OH})_2$ ?).

When some strong acid ( $\text{HClO}_4$ ,  $\text{HNO}_3$ ) is sucked into the paper before treatment with the solution, an anion chromatogram may be obtained. The sequence of anion adsorption is found to be :



This too is in accord with previous results of Schwab and Dattler.<sup>8</sup>

Weak effects of secondary anion and cation adsorption are observed. Details have been given previously.<sup>6</sup>

**2. The Molar Zone Length.**—The zone length  $h$  is a linear function of the concentration  $c$  of the ion in solution. When  $h$  is not too close to zero we may write:

$$h = h' + kc,$$

<sup>7</sup> Schwab and Jockers, *Z. angew. Chem.*, 1937, **50**, 546.

<sup>8</sup> Schwab and Dattler, *Z. angew. Chem.*, 1937, **50**, 691.

(compare Fig. 1);  $h' \approx 0.3-0.5$  mm. seems to be connected with the smaller adsorption capacity of the zone at the lower end where the paper is secured by rubber bands (cp.<sup>1</sup>).

Different ions give different zone lengths shown by the following figures.

TABLE I

Conc.		<i>h</i> in cm. for different cations								
		Cr <sup>III</sup>	Pb	Cu	Hg	Zn	Ni	Te	Mn	Ba
10 <sup>-6</sup> mole/l.	..	4.25	3.3	3.3	2.9	3.6	3.7	3.15	4.1	5.1
2 × 10 <sup>-6</sup> mole/l.	..	7.7	6.0	5.85	5.1	6.45	6.55	5.3	7.35	8.7

Considering cations of the same valency decreasing adsorption tendency seems to run parallel with increasing molar zone lengths. Increasing valency increases the molar zone length.

The length of a cation zone is to some degree influenced by the anions present in the solution as shown by the following figures.

TABLE II

	$h$ (cm.)			
	SO <sub>4</sub> <sup>2-</sup>	Cl <sup>-</sup>	NO <sub>3</sub> <sup>-</sup>	CH <sub>3</sub> COO <sup>-</sup>
Mn <sup>++</sup>	6.2	6.4	6.3	6.6
Cu <sup>++</sup>	6.4	7.7	8.0	7.1
H <sup>+</sup>	2.8	3.3	3.5	--

The effects are not very important, but they seem to a certain degree to be specific.

**3. Chromatography of Cation Mixtures.**—If we add to a solution of  $M_1$  a less strongly adsorbable cation  $M_2$ , an elongation of the chromatographic zone of  $M_1$  is observed. The reason is that a mixed zone of  $M_1$  and  $M_2$  is formed. Fig. 2 shows the relative elongation  $g = h/h_0$  as a function of the concentration ratio  $[M_1]/[M_2]$  in solution. The tendency to form a mixed zone increases with

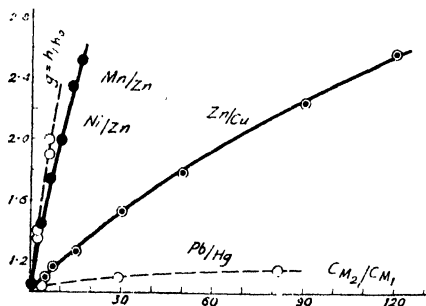


FIG. 2.—The relative elongation of the chromatographic zone as a function of the concentration ratio in the mixture.

decreasing difference in adsorption affinity. Consequently ions such as Ni and Co are completely mixed on adsorption. Only when the difference in adsorption tendency is considerable will the zone length of a cation be apparently independent of the presence of other cations. Analysis of the ion distribution in the zone shows that the ion concentration seems to be constant over the whole zone; this is also the case in an elongated mixed zone.

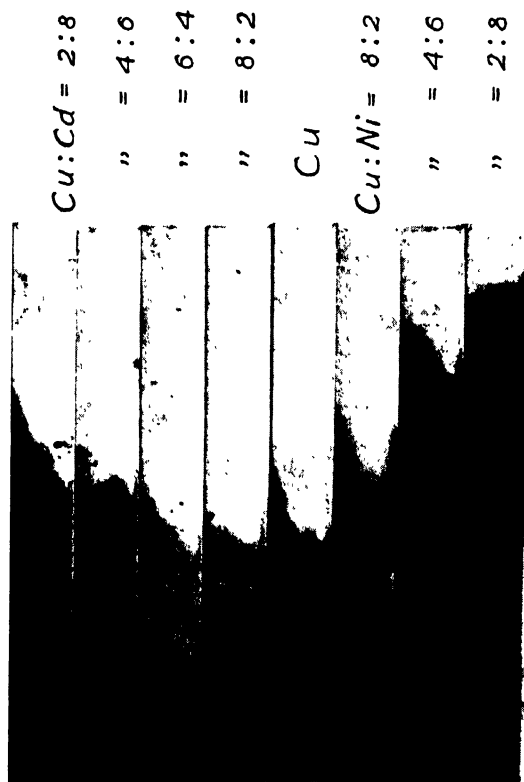
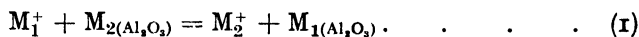


FIG. 1. - Chromatograms of Cu- Cd mixtures (developed with  $H_2S$ ) and Cu- Ni (developed with dimethylglyoxime).

$Cu + Cd + Cu + Ni = 4 \times 10^{-6}$  mole/l.



Simplified calculations show that this is in accord with the concept that the composition of the mixed zone is determined by an equilibrium of the type:



We can write:

$$K = \frac{C_{M_1}^+ a_{M_1}}{C_{M_2}^+ a_{M_2}} = \frac{C_{M_1}^+ N_2 f_2}{C_{M_2}^+ N_1 f_1},$$

where the  $C$ 's are concentrations of the original solution:  $a$ 's denote activities,  $f$ 's activity coefficients, and  $N$ 's the mole fractions of cations in the adsorbed zone.

If we assume  $f_1 = f_2$  when  $N_1 = N_2 = 0.5$  (symmetrical activity functions), it follows that  $K \simeq C_1/C_2$ , when  $h = 2h_0$ .

As only rough figures of  $K$  are required, they are not corrected for the deviations from proportionality between  $h$  and  $c$ , and for differences in molar zone length of  $M_1$  and  $M_2$ . These two approximations influence the values of  $K$  in opposite directions.

Equilibria between ions of different valency may be treated in a similar way by putting, for example,

$$K = \frac{C_{M_1}^{2+} \cdot N_{M_2} \cdot f_{M_2}}{C_{M_2}^{1+} \cdot N_{M_1}^2 \cdot f_{M_1}^2}.$$

It follows as before that

$$h \simeq C_{M_1}^{2+}/C_{M_2}^{1+},$$

where  $h_{M_2} = 2.63h_{M_1}$ .

From these experiments we can set up a scale of the adsorption affinity of cations, where the difference between two ions gives an approximate value of  $\log_{10} K$  for an exchange equilibria of the type (1). In order to have only positive numbers on the scale we give the relatively weak adsorbable  $Na^+$  ion the arbitrary value of 0.

TABLE III

---

(Na 0.0); Mn <sup>II</sup> 0.8; Ni, Co, Fe <sup>II</sup> 1.1; Cd 1.1 <sub>6</sub> ;
Zn 1.8; Cu <sup>II</sup> 3.6; Pb <sup>II</sup> 4.1; (Hg <sup>II</sup> 8).

---

It is evident from Table III that several of the common ions Mn, Ni, Co, Fe, Cd are situated close together, i.e., within 1.0 unit on the  $\log K$  scale. These ions therefore, in particular, will tend to form mixed zones.

In an earlier communication<sup>4</sup> the alkaline earth metals were included in the scale. Later experiments, however, seem to indicate that the adsorption phenomena of mixtures including these elements are more complicated than was previously thought. For example, Ba forms a mixed adsorption zone with Ni, but this is also the case with the more strongly adsorbable Cu ion.

#### 4. Chromatography of Complexes. Chromatographic Titration.—

Electrically neutral molecules or negatively charged complex ions show a negligible adsorption tendency on paper not previously treated with acid. This allows of the possibility of separating cations by addition of complex-forming reagents. When the constants of the complex formation are known, it is possible to predict the chromatographic result by simple calculation using the values of exchange constants given in §3.

This can be demonstrated by the chromatographic behaviour of glycine complexes, where the constants of the stepwise complex formation are known for some of the common ions.<sup>9</sup> Details will be published elsewhere, and only some of the results will be mentioned here.

<sup>9</sup> Flood and Lorås, *Tidsskr. kemi bergvesen metallurgi*, 1945, **3**, 83.

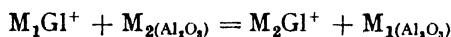


(a) **COPPER GLYCINATE COMPLEXES.** The calculations show that with a solution of  $\text{Cu/Gl/Na} = 1/1/1$ , the  $\text{CuGl}^+$  ion in contact with the adsorbent will disproportionate quantitatively into adsorbed  $\text{Cu}^{++}$  and unabsorbable  $\text{CuGl}_2$ . (Gl = glycinate ion). Therefore when a solution of  $\text{Cu}^{++}$  is added to one of  $\text{NaGl}$ , the mixture behaves as if composed of  $\text{Cu}^{++}$  and  $\text{CuGl}_2$ . This method, called chromatographic titration, can be used for a quantitative determination of copper. To a series of copper solutions are added known amounts of  $\text{NaGl}$  and the solutions are chromatographed. The equivalent amount is determined by extrapolation to zero zone length (Fig. 3). The determination is rapidly made and the accuracy obtained is better than  $\pm 5\%$ . Several other weaker complex-forming cations (such as Cd, Co, Ag, etc.) will not affect the copper determination.

(b) Corresponding calculations for Ni glycinate complexes show that the difference in affinity of Ni and Na is not large enough to give more than *ca.* 85 mole % Ni in the adsorption zone in contact with a solution  $\text{Ni/Na/Gl} = 1/1/1$ . Furthermore, with a solution of  $\text{Ni/Na/Gl} = 1/2/2$ , an appreciable amount of adsorbed Ni (10 mole %) is obtained, assuming that the adsorption tendency of  $\text{NiGl}^+$  is comparatively small.

This is in good agreement with the experimental chromatograms (Fig. 3). It is necessary to add a considerable excess of  $\text{NaGl}$  ( $\text{Ni/Gl} = 1/2.5$ ) before the adsorption of Ni becomes negligible. (The reason for this is the formation of the third complex,  $\text{NiGl}_3^-$ , which has the effect that excess of glycinate  $> 1/2$  does not increase the concentration of glycinate ions as much as in the case of copper.) Ni therefore cannot be titrated chromatographically with glycine.

(c) **CHROMATOGRAPHIC SEPARATIONS BY MEANS OF GLYCINATE COMPLEXES.** When  $\text{NaGl}$  is added to a solution of two cations  $\text{M}_1^{++}$  and  $\text{M}_2^{++}$  the result can be easily predicted. When we consider for example the equilibrium,



in a solution containing

$$C_{\text{M}_1^{++}} = C_{\text{M}_2^{++}} = 2 C_{\text{Gl}^-},$$

we find

$$\frac{N_{\text{M}_1}}{N_{\text{M}_2}} K \cdot \frac{K_{1(\text{M}_1)}}{K_{1(\text{M}_2)}} = K_0,$$

where  $K$  is the exchange constant given in § 4.  $K_1$  is the instability constant for the complex ion  $\text{MGl}^+$ .

Table IV gives the calculated values of  $K_0$  for some cation pairs. It is evident that the addition of glycinate may effect considerable changes in the normal adsorption sequence.

TABLE IV

$\text{M}_1$	$\text{M}_2$	$\log K_{1(\text{M}_1)}$	$\log K_{1(\text{M}_2)}$	$\log K$	$\log \left( K_0 = \frac{N_{\text{M}_1}}{N_{\text{M}_2}} \right)$
Cu	Zn	- 8.2	- 4.8	+ 1.8	- 1.6
Cu	Ni	- 8.2	- 5.8	+ 2.5	+ 0.1
Cu	Co	- 8.2	- 4.6	+ (2.5)	- 0.9
Cu	Cd	- 8.2	- 3.9	+ 2.5	- 1.6
Ni	Co	- 5.8	- 4.6	(0)	- 1.2
Ni	Cd	- 5.8	- 3.9	(0)	- 1.9
Co	Cd	- 4.6	- 3.9	(0)	- 0.7

After glycinate addition, Zn, Co and Cd are more strongly absorbed than Cu, and Ni is as adsorbable as Cu. These results are in good agreement with the experimental results (cp. Fig. where Cu-Co, Cu-Cd and Cu-Ni and Cu-Mg

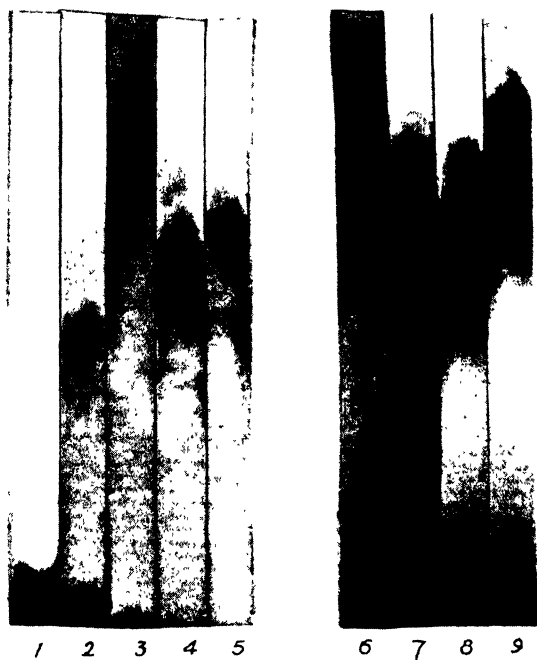


FIG. 3.

- |                         |                         |
|-------------------------|-------------------------|
| (1) Cu glycinate - 1.0  | (6) Ni glycinate - 1.18 |
| (2) Cu glycinate - 1.16 | (7) Ni glycinate - 1.20 |
| (3) Cu glycinate - 1.18 | (8) Ni glycinate - 1.22 |
| (4) Cu glycinate - 1.26 | (9) Ni glycinate - 1.24 |
| (5) Cu glycinate - 1.22 |                         |
- (1)-(5) Developed with  $K_4Fe(CN)_6$ . (6)-(9) Developed with rubane.

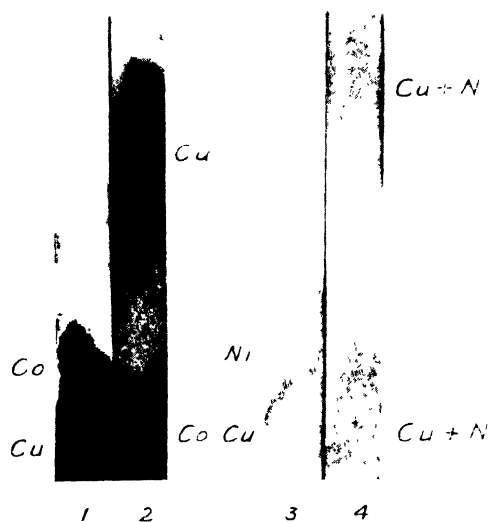


Fig. 1.

(1) Cu/Co/glycinate = 1/1/0

(2) Cu/Co/glycinate = 1/1/1

(3) Cu/Ni/glycinate = 1/1/0

(4) Cu/Ni/glycinate = 1/1/1

(1) and (2) developed with rubeane (dirty green copper zone and brown cobalt zone).  
 (3) and (4) developed with dimethylglyoxime (grey copper zone, red nickel zone).

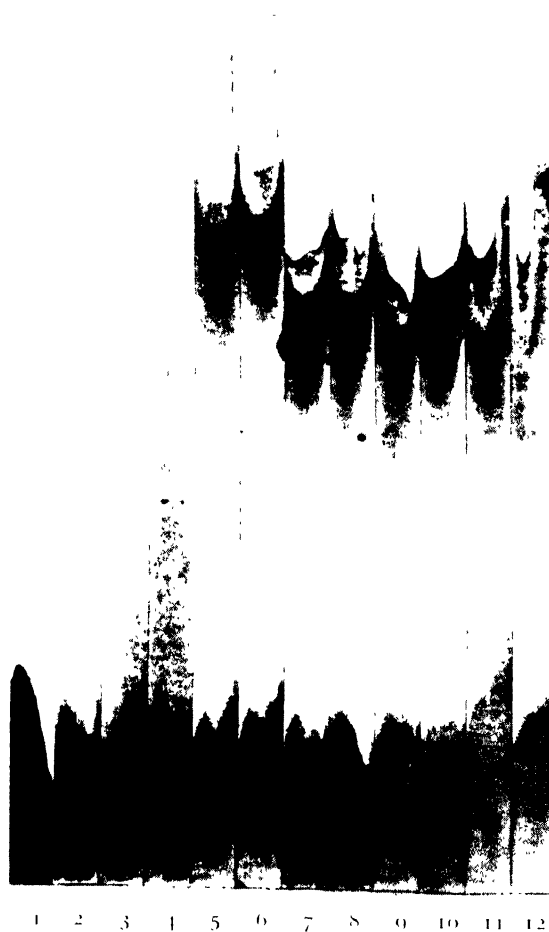


FIG. 5. Chromatograms of Ni/Co mixtures to which increasing amounts of glycine were added. The white zones at the lower end represent hydrogen ions which are formed by complex formation:  $\text{Ni}^{2+} + \text{HGl} \rightleftharpoons \text{NiGl}^{+} + \text{H}^{+}$ , etc.

The zones are developed with rubeane (brown cobalt and blue nickel zones).

The separation is very good in the strips (5)–(8) where the ratio Ni/Co/glycine is 1/1.5–10.

At larger glycine concentrations the complex formation of cobalt becomes marked.

(1) Ni/Co/glycine	1/1/0	(7) Ni/Co/glycine	1/1/9.3
(2) Ni/Co/glycine	1/1/0.6	(8) Ni/Co/glycine	1/1/11
(3) Ni/Co/glycine	1/1/1.2	(9) Ni/Co/glycine	1/1/13
(4) Ni/Co/glycine	1/1/2.4	(10) Ni/Co/glycine	1/1/15
(5) Ni/Co/glycine	1/1/5.0	(11) Ni/Co/glycine	1/1/22
(6) Ni/Co/glycine	1/1/7.0	(12) Ni/Co/glycine	1/1/44

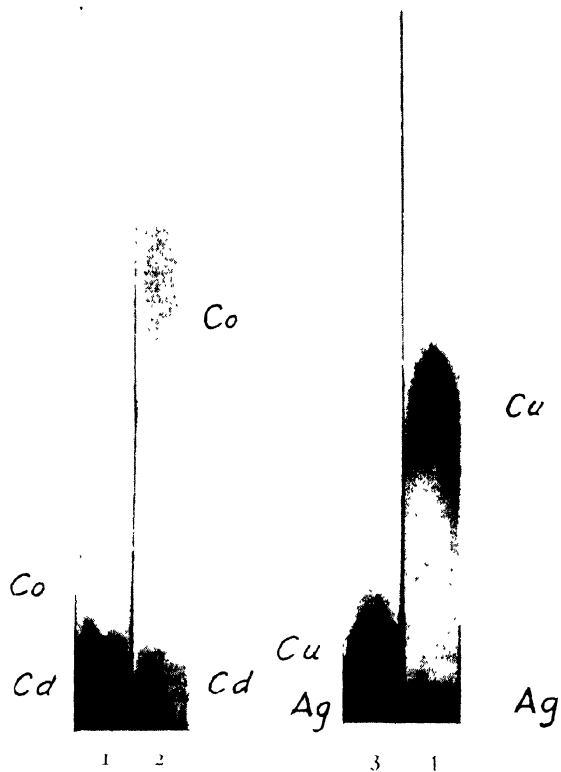


FIG. 6.—Chromatographic separation of Ag from Cu and Cd from Co by addition of glycinate (developed with  $H_2S$ ).

- |   |                              |
|---|------------------------------|
| (1) Cd/Co/glycinate :— 1/1/0                                    | (3) Cu/Ag/glycinate :— 1/1/0 |
| (2) Cd/Co/glycinate :— 1/1/1<br>(+ a little excess of glycine). | (4) Cu/Ag/glycinate :— 1/1/2 |

chromatograms are given). In the case of a Cu-Ni mixture, a mixed adsorption zone is formed, and the unadsorbed fraction also consists of both cations. In the other two cases only copper is found in the unadsorbed complex fraction. In the same way, addition of glycinate gives a good separation of Ni from Co, Ni from Cd, and Co from Cd, owing to the decreasing complex-forming tendency Ni, Co, Cd.

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## SEPARATIONS ON 8-HYDROXYQUINOLINE COLUMNS

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This paper deals with the quantitative determination of small variations in zinc content of a cupro-nickel brazing alloy by a chromatographic technique. The original table of ions separable in this manner on 8-hydroxyquinoline columns is extended, and possible further quantitative separations are discussed. Attention is also drawn to certain limitations of the method, though it is considered that it should have some popularity for certain specific quantitative applications and a wider range of appeal as a useful qualitative sorting test.

As previously noted by the present author, the technique proposed by Erlenmeyer and Dahn<sup>1</sup> of separating inorganic ions on a column composed of 8-hydroxyquinoline and starch was used to determine quantitatively small variations in zinc content of a cupro-nickel-zinc alloy.<sup>2</sup> The method consisted in adsorbing the metals (sample weight 2-6 mg.) from sulphate solution buffered to pH 5-6 with sodium acetate. Tubes of suitable size were found to be 10 cm. long by 5 mm. internal diam. Starch and 8-hydroxyquinoline in equal amounts by weight were well mixed and ground to pass a 60-mesh sieve and be retained on one of 80 meshes to the linear inch. Packing was effected by tapping the tube on the bench after each small addition of adsorbent; tamping was found most unsatisfactory because of uneven pressures giving rise to slow filtration and unsatisfactory banding. Whilst dilution of the above sample weight to a concentration less than 2 mg./ml. proved to have no deleterious results, higher concentrations were found occasionally to give somewhat unsatisfactory banding. In this connection, the most frequent trouble was co-precipitation of the green fluorescing zinc complex along with the copper band (green in ordinary light) at the top of the tube. After adsorption of the coloured complexes, washing was most satisfactorily effected with 5 % sodium acetate solution. Dilute aqueous acetic acid as sometimes advocated has always seemed to promote undue channelling of the column before any effect on the banding could be seen. As in the chromatography of organic compounds using alumina columns, it was found to be important that the top of the column be never allowed to become free of liquid either during addition of the metal solution or the final washing. A suitable amount of washing liquid would seem to be three to four times the volume of the initial solution.

<sup>1</sup> Erlenmeyer and Dahn, *Helv. chim. Acta*, 1939, **22**, 1369; 1941, **24**, 878.

<sup>2</sup> Robinson, *Metallurgia*, 1947, **37**, 45, 107.

Quantitative measurements were obtained from the length of the appropriate band as compared with the lengths of corresponding bands obtained at the same time and under precisely the same conditions as the unknown sample, but using a solution containing accurately known amounts of the metals concerned. Where unusually small amounts of metallic ions had to be determined it was found advisable to reduce the diameter of the column of adsorbent in order to allow the formation of a measurable length of coloured band. The colours of the respective bands of the three metals were (in descending order down the tube): copper, dark green; nickel, yellow; zinc, yellow. Fortunately, only the zinc complex was fluorescent in ultra-violet light so that its zone length could easily be measured.

As foreshadowed in the previous report on the above work, it was hoped to utilize the 8-hydroxyquinoline technique as a means of identification and possible determination of the constituents of alloy steels. It was soon found, however, that the large amount of iron present in these alloys constituted a very serious difficulty because the whole of the column became stained with the ferric complex, so that virtually only one large black band existed. Various attempts were made to restrict the iron band to its correct position on the column (i.e., below the yellow zinc zone). The first line of attack was to find an optimum pH which would allow of a sharp separation, but no success was achieved within the range pH 3–8. This appeared the most likely range as below pH 3 the mobility of the iron might have been sufficient to enable it to be washed out of the column, but the other bands were far too imperfect, due to channelling, to allow of identification. Above pH 8, all that could be seen was a single heavy black zone of iron, which presumably contained everything else, even where complexing of the iron with tartrate was used to prevent its prior precipitation as the hydrate.

So far, the only conditions under which any other complexes than iron could be seen (and even then measurement was virtually impossible, so prohibiting quantitative work) have been achieved by reduction of the iron to the ferrous condition with hydroxylamine hydrochloride. In this circumstance the ferrous complex appeared as a faint greenish-grey band occupying almost the whole of the column, but above which could be seen a green band due to Mn, a yellow band due to Ni, and an orange band due to Mo in that order, when using nickel steels containing from 0.5 % to 3.5 % nickel and up to 0.5 % molybdenum. With a plain carbon steel containing 0.25 % vanadium, the grey vanadium zone was seen immediately above the manganese band.

Even these rather disappointing results were only obtained by packing the column for a distance of about 2 cm. above the 8-hydroxyquinoline-starch mixture with powdered hydroxylamine hydrochloride in order to maintain adequate reducing conditions: again, the most satisfactory pH range appeared to be about 5–6, though if one was prepared to sacrifice some definition of the manganese zone in ordinary low-alloy steels, and of the tungsten zone in high-speed steels, slightly better results were obtained at pH 3 (minimum). High-speed steels did not seem very satisfactory materials, owing to the need for either complexing the iron, to allow work at high pH values, or alternatively complexing the tungsten, for working at the other end of the scale. Both methods gave rise to indeterminate banding and doubtful colours.

8-Hydroxyquinoline columns have been used with far greater success in non-ferrous analysis and, provided one only attempts to use alloys of simple composition, the method has definite value for determining local variation in composition or rapid identification of extraneous metals, especially where one is limited as to amount of sample available. It has been possible to

determine the stability of silver in a brazing alloy on brazing, by a method similar to that reported elsewhere for zinc. The alloy concerned contained 89 % copper, 2 % silver and 9 % zinc. Silver produced a yellow band above the green copper zone when working at pH 3.5 to 4.5, which appeared the most suitable range, though to prevent channelling a 40 % oxine-60 % starch column had to be used instead of the normal 50/50 mixture. That the upper yellow band was free from possible contamination with co-precipitated zinc was verified by the fact that only a faint yellow fluorescence in ultra-violet light was observed whereas the lower zinc zone showed its familiar bright green fluorescence. At higher pH values than 5 some co-precipitation takes place and quantitative work is impossible. However, in no case where silver was absent has precipitation of the zinc above the copper been noticed, so it would seem that an upper yellow line or band may, in a cupro-zinc alloy, be taken as a suggestion of the presence of silver. One important observation noted in all experiments with brazing alloys after use was that contamination of the metal with iron during sampling was immediately evident by a comparatively broad band of the black ferric hydroxyquinolate complex appearing beneath the coloured non-ferrous zones. Most samples showed a thin black line at this position, rarely more than 0.5 mm. thick, and it was considered that this was due solely to diffusion of iron from the two metals being joined into the brazing alloy. The band referred to above as being due to contamination in sampling was always considerably greater than this and was seldom less than 2 mm. in length.

Results obtained for the two types of brazing alloy considered here, i.e., copper-nickel-zinc and copper-zinc-silver, in which only zinc and silver respectively were considered, were as follows, one typical case only for each type of alloy being quoted. Both cases were calculated by comparison with a solution in nitric acid and buffered to the appropriate pH with sodium acetate, containing 2 mg. of the "as received" alloy per ml. In some cases, better results for the cupro-nickel alloy have been obtained, however, by replacing the nitric acid by sulphuric before the addition of sodium acetate.

#### *Copper-nickel-zinc alloy*

Initial zinc content	..	..	..	21.75 %
Final	"	"	1st bar	16.50 %
"	"	"	2nd "	14.50 %
"	"	"	3rd "	14.00 %
"	"	"	4th "	12.50 %

#### *Copper-zinc-silver alloy*

Initial silver content	..	..	..	2.18 %
Final	"	"	1st bar	2.00 %
"	"	"	2nd "	1.75 %
"	"	"	3rd "	1.9 %
"	"	"	4th "	1.75 %
"	"	"	5th "	2.00 %
"	"	"	6th "	1.8 %

The use of a chromatographic adsorption technique on columns of 8-hydroxyquinoline would seem from the foregoing to be a useful method of dealing with the type of problem mentioned, where one is concerned with the sampling and ultimate analysis of the thin line of metal left at a brazing joint. In those cases quoted it rendered possible the required analysis without the necessity for destruction of the finished component. Even in those cases where the final product could be damaged completely, only a few milligrams of sample free from contamination with the surrounding ferrous material were available, and a micro or semi-micro technique would have been



necessary in any case. Nevertheless, the method has severe limitations, particularly in the field of analysis of ferrous materials. In the non-ferrous field the greatest problem would appear to be that of distinguishing between complexes, due to the fact that so many are yellow in colour, and none except zinc and magnesium have distinguishing fluorescence colours.

Other difficulties seem to be largely of a mechanical nature such as diffuse banding and channelling. In the opinion of the present author these faults would seem largely due to the solvent action of either the initial solution or the wash liquid on the oxine of the column. This would seem to prevent the use (so often suggested) of dilute acetic acid as a developing medium. The writer has attempted many times, especially with samples of ferrous materials, to use this method of development but without success owing to the production of comparatively large holes in the side of the column and the consequent mechanical displacement of the zones already produced. There is no doubt whatever that the black iron complex, so devastating in work with ferrous materials, could be washed through the column before the other complexes with about 5 % acetic acid were it not for the fact that the column itself is literally washed through as well.

The use of ammonia is complicated also by its solvent action on oxine and, since so many metallic oxine precipitates are remarkably stable at high pH values, it would seem hardly likely that this reagent would promote more satisfactory banding, except possibly with iron, tungsten and molybdenum. Thus, it appears fairly certain that the optimum pH value for work with columns of this reagent must be very close to the neutral point.

As a result of the previously mentioned experimental work it would now seem possible tentatively to extend the original order of separation on 8-hydroxyquinoline columns as suggested by Erlenmeyer and Dahn to include also manganese, silver and molybdenum, whose positions have been ascertained with some degree of certainty. Additionally, aluminium and magnesium can be added to this list in approximate positions as a result of attempts to use their distinctive fluorescence colours to separate molybdenum and cobalt, when fixing the position of the former metal, the order of separation now given being :

$\text{VO}_3^{--}$	.. ..	grey
$\text{Mn}^{++}$	.. ..	green
$\text{WO}_4^{--}$	.. ..	yellow
$\text{Ag}^+$	.. ..	yellow
$\text{Cu}^{++}$	.. ..	green
$\text{Bi}^{++}$	.. ..	yellow
$\text{Ni}^{++}$	.. ..	yellow
$\text{MoO}_4^{--}$	.. ..	orange
$\text{Al}^{+++}$	.. ..	green (bright green fluorescence)
$\text{Co}^{++}$	.. ..	reddish
$\text{Zn}^{++}$	.. ..	yellow (bright green fluorescence)
$\text{Mg}^{++}$	.. ..	green (light blue fluorescence)
$\text{Fe}^{+++}$	.. ..	black
$\text{UO}_2^{++}$	.. ..	orange

Further extension of this list appears improbable unless some satisfactory method of separating the yellow complexes can be devised.

The author is indebted to the directors of Messrs. W. Fearnough Ltd. for permission to publish the work mentioned here.

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## THEORY OF CHROMATOGRAPHY

### VIII. The Separation of Lithium Isotopes by Ion Exchange and of Neon Isotopes by Low-temperature Adsorption Columns

BY E. GLUECKAUF, K. H. BARKER AND G. P. KITT

*Received 15th August, 1949*

An essential condition for the successful separation of isotopes (or of any substances of very similar sorption characteristics) is that the chromatographic boundaries between the species to be separated remain as sharp as possible. The effects of such experimental features as grain size, flow-rate, concentration, pore space, diffusion constants in the liquid and in the sorbent phase are discussed.

Preliminary experiments to test these conceptions have been made by separating (a) lithium isotopes by ion-exchange columns and (b) neon isotopes by low-temperature adsorption columns. Though in the case of lithium almost pure  $^7\text{Li}$  has been obtained, the quantity of material enriched has been far less than expected. A similar observation was made by Taylor and Urey in 1938.

In the case of neon separations the effect of variations in experimental conditions on the enrichment is well represented by the theoretical equations, and the absolute values of the "theoretical plate" height calculated from the observations agree with the theory.

### Symbols

- $a$  = numerical factor connected with the form of the grains; for spherical grains =  $1/4\pi^2$ .
- $A$  = g. sorbent per cm. of column length =  $\bar{x}/L$ .
- $c$  =  $\varphi(q)$  = the concentration of the solute, or in case of two solutes, of the following solute (mole/l.).
- $C$  = the total concentration of all solutes.
- $d$  = grain diameter (in cm.).
- $D_s$  and  $D_l$  = diffusion coefficients of the solute in the solid sorbent and in the liquid phase respectively.
- $D_{\text{Ne}}$  = self-diffusion coefficient of neon.
- $f(c)$  =  $f^*(c) + \alpha c$  = amount of solute per g. sorbent including the pore space.
- $F$  = eluate velocity in ml. per sec.
- $L$  = length of the whole column (in cm.).
- $q^*$  = amount of solute adsorbed when not in equilibrium with solution.
- $Q$  = total amount of all solutes adsorbed per g. sorbent.
- $s$  = density of sorbent material.
- $t$  = time (sec.).
- $v$  = volume of eluate from bottom of the column (in ml.).
- $V_t$  = threshold volume =  $Q \cdot \bar{x}/C$ .
- $x$  = distance from top of column (in g. sorbent).
- $\bar{x}$  = length of column (for elution experiments) or otherwise: length of column passed by the frontal band, i.e., position of the front of solute 1 =  $Cv/Q$  (in g.).
- $\bar{\bar{x}}$  = hypothetical position of the sharp front of solute 2, if no disturbances were active.
- $z$  = distance from top of column (in cm.).
- $\alpha$  = pore space in ml. per g. sorbent.
- $\delta$  = thickness of laminar solvent layer through which diffusion takes place.
- $\Delta$  = disturbance factor, representing all disturbances:  $\Delta/2A$  corresponds to the height of a "theoretical plate."

$\square$  = free cross-sectional area of the column through which the solvent flow passes.

$\eta$  = relative enrichment =  $\frac{c^{\circ} - c}{c^{\circ}}$ .

The superscript  $^{\circ}$  refers to the original concentration of a solute. The subscripts  $_1$  and  $_2$  refer to the less and more adsorbed solute respectively.

In the theory of chromatography it has become customary to introduce simplifying assumptions which render it possible to deal quantitatively with certain aspects of chromatographic procedure. In order to study the effects of the adsorption isotherm on the distribution of solutes in the column it has been necessary to assume that the grain size of the adsorbent is infinitely small and that no disturbing effects due to diffusion or non-equilibrium occur in the column. On the other hand, where such disturbing effects have been treated workers have usually dealt only with a linear isotherm, for which it is possible to solve the differential equation, although certain features occurring with the normal non-linear isotherms are lost.

The case of non-equilibrium due to grain-diffusion has been discussed for non-linear isotherms, but the discussion was restricted to the effect on self-sharpening front boundaries, such as occur in frontal analysis and displacement development (see Tiselius<sup>1</sup>). The reason for this was that these boundaries reach, after an initial period, a final shape and gradient, where the disturbing effects and the self-sharpening tendency due to the curved isotherm just balance each other. This final shape is accessible to calculations.

Table I gives a survey of these partial problems and of the authors who have dealt with them, and at the same time shows the gaps which still exist in our present knowledge.

The main gap in our knowledge is connected with the effect of disturbances on the shape of the rear boundary of chromatograms in the case of non-linear isotherms. This problem depends on the solution of differential equations of the type:

$$\left(\frac{\partial f(c)}{\partial v}\right)_x + \left(\frac{\partial c}{\partial x}\right)_v - \Delta \left(\frac{\partial^2 c}{\partial x^2}\right)_v = 0 \quad (\text{Disturbance type A, B}), \quad (1)$$

$$\text{and} \quad \left(\frac{\partial f(c)}{\partial v}\right)_x + \left(\frac{\partial c}{\partial x}\right)_v + \Delta' \left(\frac{\partial^2 c}{\partial x \partial v}\right) = 0 \quad (\text{Disturbance type c}), \quad (2)$$

which has so far not been possible, except in the case of linear isotherms.

The recent developments of the theory, however, make it possible to discuss with confidence the influence of some experimental factors on the efficiency of chromatographic separations. Such factors are grain size, flow rate, temperature, diameter of column, uniformity of packing and quantity of sorbent. The last two need not be discussed here. The need for uniform packing has been stressed from the very beginning of the chromatographic technique. The quantity of sorbent required for complete separation is a function of the quantity of the solutes to be separated and of the multiple adsorption isotherm (see<sup>2 3 4</sup>).

<sup>1</sup> Tiselius, *Arkiv. Kemi, Min. Geol. A*, 1943, 16, No. 5, paper 18.

<sup>2</sup> Glueckauf, *Proc. Roy. Soc. A*, 1946, **186**, 35.

<sup>3</sup> Coates and Glueckauf, *J. Chem. Soc.*, 1947, 1308.

<sup>4</sup> Glueckauf, *J. Chem. Soc.*, 1947, 1321.

The influence of the other four factors is somewhat complex and it is an advantage to deal rather with the fundamental phenomena of

- (i) effects of finite grain size,
- (ii) diffusive mixing in longitudinal direction,
- (iii) non-equilibrium (due to slow diffusion in the particles),
- (iv) non-equilibrium (due to slow diffusion in the liquid).

The best way to demonstrate the effect of these phenomena on the efficiency of separation is to discuss their effect on the gradient of self-sharpening front boundaries between two solutes for the case of displacement development.

TABLE I  
SURVEY OF THEORETICAL LITERATURE

		Ideal Column	Disturbing Effects
			A Grain size B Diffusion C Non-equilibrium (grains) D Non-equilibrium (solution)
Linear Isotherm	{ Single Solute	(5)	A (6) (7) D (8)
	{ Two Solutes	(5)	A (7) this paper
Non-linear Isotherm	{ Single Solute	(9) (10) (11)	C : (12), A, B, C, D : this paper ; discussion limited to front boundary. Diffuse rear boundaries : unsolved
	{ Two Solutes	(9) (2) (3) (4) (13)	A, B, C, D : this paper ; discussion limited to frontal interboundary. Diffuse rear boundaries : unsolved
Calculation of non-linear isotherm from elution curves		(9) (14) (15) (16)	A, B : (17)

**A. Boundary Considerations for the Case where a Frontal Band of Pure Solute 1 has been Formed.**—It is apparent in displacement development where the separated bands do not move away from each other that the efficiency of the final separation depends entirely on the width of the separation zone, which itself is dependent on the gradient of the boundaries.

For the gradient  $dc/dx$  in the column and  $dc/dv$  of the elution curve of the slower of the two solutes we obtain the following equations.

In the case of the disturbance phenomena (i), (ii), (iii) :

$$-\left(\frac{\partial x}{\partial c}\right)_v = \frac{c^0}{f(c^0)} \left(\frac{\partial v}{\partial c}\right)_x = \frac{(K_1 \text{ or } K_2) + K_3}{c^0 f(c)/f(c^0) - c} \quad (3)^*$$

<sup>5</sup> Wilson, *J. Amer. Chem. Soc.*, 1940, **62**, 1583.

<sup>6</sup> Martin and Syngé, *Biochem. J.*, 1941, **35**, 1385.

<sup>7</sup> Mayer and Tompkins, *J. Amer. Chem. Soc.*, 1947, **69**, 2866.

<sup>8</sup> Boyd, Myers and Adamson, *J. Amer. Chem. Soc.*, 1947, **69**, 2836, 2849.

<sup>9</sup> De Vault, *J. Amer. Chem. Soc.*, 1943, **65**, 532.

<sup>10</sup> Weiss, *J. Chem. Soc.*, 1943, 297.

<sup>11</sup> Glueckauf, *J. Chem. Soc.*, 1947, 1302.

<sup>12</sup> Glueckauf and Coates, *J. Chem. Soc.*, 1947, 1315.

<sup>13</sup> Glueckauf, This Discussion.

<sup>14</sup> Weil-Malherbe, *J. Chem. Soc.*, 1943, 303.

<sup>15</sup> Glueckauf, *Nature*, 1945, **156**, 748.

<sup>16</sup> Glueckauf, *Nature*, 1947, **160**, 301.

<sup>17</sup> Glueckauf, *J. Chem. Soc.*, 1949, 3280.

\* Both  $K_1$  and  $K_2$  take account of mixing effects in more or less the same space so that the larger of the two ought to be used only.

and in the event of (iv) :

$$= \frac{K_4}{c - \varphi\left(\frac{c}{c^0} \cdot f(c^0)\right)} \quad (4)$$

As is shown in the appendix in detail, one can derive for the value of the constants,

$$K_1 = \frac{1}{2} dA \quad (3a)$$

$$K_2 = \frac{\alpha D_t A^2}{\sqrt{2} F} \quad (3b)$$

$$K_3 = \frac{ad^2 F f^*(c^0) c^0}{D_s (f(c^0))^2} \quad (3c)$$

$$K_4 = \frac{\delta d F s}{6 D_l} \quad (4a)$$

where for turbulent flow  $\delta$  is proportional to  $1/F$  and for laminar flow (and small grain sizes)  $\delta \simeq ad$ .

In the case of isotope separation the variable boundary functions (in the denominator) become simplified and identical, and we obtain

$$-\left(\frac{\partial c}{\partial x}\right)_v = \frac{f(c^0)}{c^0} \cdot \left(\frac{\partial c}{\partial v}\right)_x = \frac{(K-1)}{\Delta} \cdot \frac{c_2}{C} \cdot (c_2^0 - c_2) \quad (5)$$

where  $C$  is the total concentration of both isotopes and  $c_2$  that of the slower isotope,  $K$  is the separation factor and  $\Delta = (K_1 \text{ or } K_2) + K_3 + K_4$ . In the case of ion exchange  $K$  is the equilibrium constant, and for adsorbents it is the ratio of the adsorption coefficients of the two isotopic solutes.

Eqn. (5) shows that, while boundary phenomena may be of secondary importance in the separation of compounds with large separation factors, they attain a first-rate importance, when  $K$  is small. Except for H, He and Li isotopes, separation factors are always less than 1.01.

If numerical values are inserted into eqn. (3) and (4) one can see that for different types of chromatographic technique different terms will be predominant. In *ion exchange*, the predominant term is usually due to *non-equilibrium* ( $K_3$  or  $K_4$ ), though we have been able to reduce grain size and flow speed to such an extent that the diffusion term  $K_2$  becomes dominant, which results in a not very pronounced optimum as regards sharpness of the boundaries. An increase of temperature, increasing the values of  $D_s$  (and  $D_l$ ), is, as a rule, beneficial in the case of dominant non-equilibrium, and this is borne out by experiments (see <sup>17, 18</sup>).

In the chromatography of *organic substances* on impermeable adsorbents, where adsorption equilibrium is almost instantaneous, the predominant term is due to the effect of finite grain size  $K_1$ . Thus the separation of substances which are very difficult to separate should therefore be considerably improved by reduction of the grain diameter.<sup>13</sup> Accordingly one would expect here little effect (within limits) from variations of flow velocity; this corresponds to general experience. The effect of the diameter of the column and of the particle size, in this case, was well shown by Weil-Malherbe<sup>14</sup> (Fig. 3 and 4), and whilst his experiments (non-linear isotherm + disturbance + complete band development) cannot be interpreted quantitatively, the effect observed corresponds to what one would expect from eqn. (3a).

Apart from excessively low flow velocities it is only in *gas chromatography* and possibly in partition chromatography on a paper adsorbent that the

diffusion term attains major importance and here the flow velocity cannot be reduced beyond certain limits. In the first case a reduction of temperature should be of double benefit, since not only will the separation factor be increased, but  $D_l$  (and  $D_s$ ) will also be reduced.

**DIAMETER OF COLUMN.**—This is defined by the factor  $A$  (g. adsorbent per cm. of column) which enters  $K_1$  and  $K_2$ . It is therefore important in those cases where the finite grain size effect is predominant, e.g., in the separation of organic substances by surface-active sorbents. A reduction of the tube diameter combined with increased length will therefore be an advantage. This corresponds to the general practice when difficultly separable substances are concerned.

### B. Frontal Phenomena in the Case of Partial Enrichment only.

—In most cases of isotope separation a frontal band of a pure species does not usually appear but only smaller or larger enrichments of the less adsorbed isotope are achieved. In this case the form of the boundary is not given by eqn. (5) which applies only for the "final" state, i.e., after appearance of an S-shaped boundary and of a pure frontal band. If there is only moderate enrichment, eqn. (1) for the more strongly adsorbed isotope  $c_2$ , if this is only as a small proportion of the total concentration  $C_1$ , becomes

$$\frac{QK}{C} \cdot \left( \frac{\partial c_2}{\partial v} \right)_x + \left( \frac{\partial c_2}{\partial x} \right)_v - \Delta \left( \frac{\partial^2 c_2}{\partial x^2} \right)_v = 0, \quad (6)$$

as

$$f(c_2) = \frac{QK}{C} \cdot c_2 / (1 + (K - 1) \cdot c_2 / C), \quad (7)$$

where  $(K - 1)c_2/C \ll 1$  and can be neglected for  $c_2 \ll C$ .

Here  $Q$  is the total amount adsorbed per g. adsorbent in equilibrium with  $C$ ;  $C$  is the total concentration of both isotopes;  $K$  is the separation factor.

$$K = \frac{f(c_2) \cdot (C - c_2)}{(Q - f(c_2)) \cdot c_2} \text{ and } \bar{x} = v Q / C.$$

In the following the subscript 2 is dropped. The boundary conditions are  $c = c^0$  for  $v = 0$ ,  $x < 0$ ,

$$\text{and } \frac{QK}{C} \int_0^{\bar{x}} (c_0 - c) \cdot dx = v \cdot c_0 \cdot (K - 1),$$

which is a condition for the conservation of mass of solute 2 within the boundaries of the total solute band. Instead of  $\int_0^{\bar{x}}$  we can write with an almost infinitesimal error  $\int_{-\infty}^{\bar{x}}$ . The solution for eqn. (6) under these conditions has been obtained by Dr. B. Davison (A.E.R.E.):

$$\begin{aligned} \frac{c}{c^0} = & \frac{1}{2} \left[ 1 - \operatorname{erf} \left( \frac{x - \bar{x}}{2 \sqrt{\bar{x} \Delta}} \right) \right] - (K - 1) \sqrt{\frac{\bar{x}}{\Delta \pi}} \cdot \exp \left\{ - \frac{(x - \bar{x})^2}{4 \bar{x} \Delta} \right\} + \\ & \frac{1}{2} \left[ 1 - \frac{(K - 1)(x - \bar{x})}{\Delta} + 2(K - 1)^2 \frac{\bar{x}}{\Delta} \right] \left[ \exp \left\{ (K - 1)^2 \frac{x}{\Delta} - (K - 1) \frac{(x - \bar{x})}{\Delta} \right\} \right] \\ & \times \left[ 1 + \operatorname{erf} \left( \frac{(x - \bar{x}) - 2(K - 1)\bar{x}}{2 \sqrt{x \Delta}} \right) \right] \quad (7) \end{aligned}$$

where  $\bar{x} = \bar{x}/K$ , which is the point where the front boundary of solute 2 would be in the case of  $\Delta = 0$ , i.e., if there were no disturbance. For

values in the vicinity of the front boundary in the case of small enrichment, i.e., for  $\bar{x} < \Delta/(K-1)^2$  this equation simplifies to

$$\frac{c^\circ - c}{c^\circ} = 2(K-1)\sqrt{\bar{x}/\Delta\pi} - (K-1)^2\bar{x}/2\Delta - (K-1)(\bar{x}-x)/\Delta. \quad (8)$$

Eqn. (8) gives the possibility of calculating both  $K$  and  $\Delta$  from the experimentally observed slope and the enrichment at the front boundary. Using the symbols  $\eta$  for the relative change of the isotope ratio and  $S$  for the gradient of the latter with respect to  $x$ , both referring to the front of the band, we obtain

$$\bar{\eta} = \left[ \frac{c^\circ - c}{c^\circ} \right]_{\bar{x}} = 2(K-1)\sqrt{\bar{x}/\Delta\pi} - (K-1)^2\bar{x}/2\Delta, \quad (9)$$

$$S = \left[ \frac{\partial \left( \frac{c^\circ - c}{c^\circ} \right)}{\partial x} \right]_{V, \bar{x}} = \frac{K-1}{\Delta},$$

or in the case of eluted solutes

$$S' = \left[ \frac{\partial \left( \frac{c^\circ - c}{c^\circ} \right)}{\partial v} \right]_{V, \bar{x}} = \frac{C}{Q} \cdot \frac{(K-1)}{\Delta}. \quad (10)$$

Solving for  $(K-1)$  and for  $\Delta$  (whereby  $\bar{x}$  in eqn. (9) can be considered equal to  $\bar{x}$ ) gives

$$(K-1) = \frac{\pi\bar{\eta}^2}{4vS'} \left( 1 + \frac{\pi\bar{\eta}}{4} + \frac{5}{64}\pi^2\bar{\eta}^2 \dots \right), \quad (11)$$

$$\Delta = \bar{x}\pi \left( \frac{\bar{\eta}}{2vS'} \right)^2 \left( 1 + \frac{\pi\bar{\eta}}{4} + \frac{5}{64}\pi^2\bar{\eta}^2 \dots \right), \quad (12)$$

where the last term in the bracket is no longer of importance.

If  $K$  is known, it is possible to calculate  $\Delta$  from any observation near the front boundary by transforming eqn. (8) into

$$\Delta = \left[ \frac{4(K-1)^2\bar{x}}{\eta^2\pi} \right] - \left[ (K-1)^2 \frac{\bar{x}}{\eta} \right] - \frac{2(K-1)\bar{x}(v-V_l)}{\eta V_l}. \quad (13)$$

**C. Application to the Separation of Isotopes.**—It is observed from eqn. (5) that, in the case of isotopes, due to the small factor  $(K-1)$ , the enrichment zone extends greatly in length and becomes the most important part of the chromatogram; moreover in the case of isotopes a frontal band of pure species is not usually obtained. For a better understanding, it is useful to draw a comparison between this enrichment zone and a distillation column.

Both are based on the equilibrium distribution of two or more substances between two phases. In distillation these phases are liquid and gas, in chromatography they are liquid and solid. In the first case we have two mobile fluids, which easily lend themselves to a counter-current process owing to their physical characteristics. This counter-current leads to a cascading of the single-stage equilibrium enrichment.

In chromatography we have only one fluid. In order to produce a counter-current, it is necessary, as the solid phase cannot move, that the "fractionation column" is shifted with respect to the solid phase, i.e., the bands of solutes pass through the column. The parallelism, between distillation and chromatography becomes complete, if we consider the

enrichment-zones of the chromatographic band as "mobile fractionation columns." If the observer travels along with the enrichment zone in such a way that he always stands at the sharp front boundary, then the situation is exactly analogous to a distillation column with the observer standing at the reflux head. There are many similarities in the equations and  $\Delta/2A$  in eqn. (1) corresponds to the height of a "theoretical plate" in a distillation column.

### Experimental

**Experiment on the Separation of the Lithium Isotopes.**—In order to obtain the smallest possible value of  $\Delta$  experiments were made using :

- (a) an ion-exchange resin, Zeo-Karb H.I., for which a diffusion coefficient of about  $10^{-6}$  cm.<sup>2</sup>/sec. was obtained (faster ion exchangers are now available);
- (b) a fairly uniform grain size of about  $1.5 \times 10^{-3}$  cm. diam., which was obtained by grinding and subsequent fractionation by air elutriation;
- (c) a flow-rate of only  $4 \times 10^{-4}$  ml./sec.

Other experimental data were :

Amount of exchanger, 45 g. =  $\bar{x}$ .

Original solution, N lithium acetate.

Threshold volume, 162 ml. =  $\left(\frac{Q}{C} \cdot \bar{x}\right)$ .

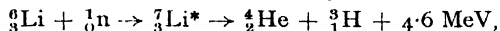
Pore space ratio ( $\alpha$ ), 0.3 ml./g. exchanger.

Length of column, 90 cm. =  $\bar{x}/A$ .

Samples of  $\frac{1}{3}$  ml. (= 3 drops) were collected and were analyzed for isotopic composition.

Several runs were made with a number of identical tubes, and it could be observed that by far the most important factor influencing the efficiency of separation was any distortion of the horizontal boundary. Inclined and serrated boundaries give rise to lateral diffusion, the effect of which cannot be assessed quantitatively. As the column of the Zeo-Karb changes to a paler hue at the front boundary of the Li acetate solution, this feature could be checked visually. Only one run gave a perfectly straight and horizontal boundary and the data of this run are discussed below in detail. As would be expected the other runs gave much lower changes of the isotopic ratio in the eluted solution.

**Determination of the Isotopic Ratio of Lithium.**—A nuclear reaction was used for the isotopic analysis. When  ${}^6\text{Li}$  is bombarded with slow neutrons, heavy ionizing particles are emitted according to the reaction :



while no comparable reaction occurs with  ${}^7\text{Li}$ .

The ranges for the disintegration products in air are 1.5 cm. for the  ${}^4\text{He}$  and 5.5 cm. for  ${}^3\text{H}$  particles. As the two particles are emitted in opposite directions the chances that one of them enters the ionizing chamber and produces an ionization sufficient for triggering the counting device are very large. Thus the number of disintegration particles counted under neutron irradiation gives a convenient measure of the relative amount of the lighter isotope. By reference to a standard of unseparated lithium salt with 7.5 %  ${}^6\text{Li}$ , these observations can be expressed as percentage enrichments or depletions.

After some preliminary experiments it was decided to employ an air-filled ionization chamber in preference to either a fast, argon-filled chamber or a proportional counter. This method has the advantage of simplicity and reliability of operation and greatly facilitates the changing of specimens. The efficiency of detection of the disintegration particles can be made greater than in the other method by actually incorporating the specimens in the chamber. As shown in Fig. 1 the target disc D constituted one plate of the ion chamber.

To minimize the background due to  $\gamma$ -rays from the Ra-Be neutron source, the volume of the chamber was kept as small as possible compatible with a good signal to noise ratio. The chamber was of the parallel plate type and



had a cylindrical guard ring  $G$  surrounding the collecting electrode  $E'$ . Polythene insulation was used and the outer cylindrical cover  $E$  was connected to the H.T. electrode. This cylindrical shape facilitated the insertion of the chamber in a paraffin block employed to slow down the fast neutrons. The electrode spacing was 4 mm. and the applied H.T. voltage 2000 V, giving a collecting field of about 5000 V/cm. throughout the series of experiments. The effective volume of the chamber was about 1.5 cm.<sup>3</sup>.

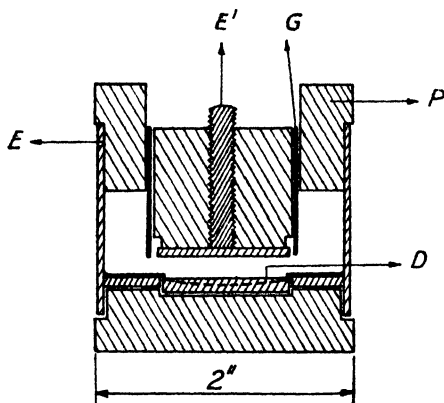


FIG. 1.—Ion chamber for observing the particle emission from  $^6\text{Li}$  during neutron irradiation.

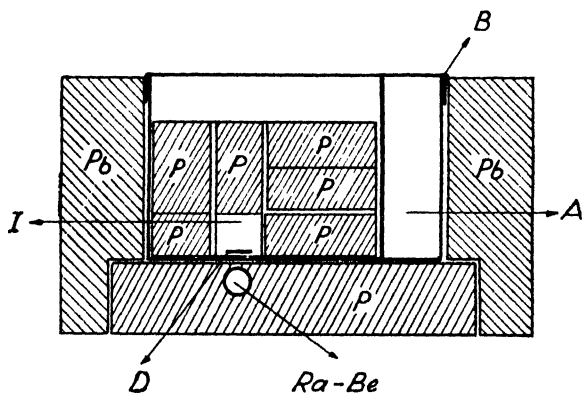


FIG. 2.—Irradiation arrangement.  
I = Ion chamber.

Fig. 2 shows the dispositions of Ra-Be source, paraffin moderator  $P$  and lead shielding  $Pb$ . At least 10 cm. paraffin surrounded the source in every direction. The radium was pushed into a lead tunnel when the specimens were changed by hand. No lead was required between the source and chamber to cut down  $\gamma$ -radiation, which were at out 2–3 cm. apart.

For convenience the pre-amplifier  $A$  was contained in the same screened box  $S$  as the ion chamber, to keep the effective collecting capacity at a low value. This was followed by a five-stage resistance-capacity coupled amplifier of conventional design, with a negative feedback. The filaments of the amplifier were supplied in series from a stabilized source of D.C. in an attempt to improve the gain stability.

The characteristics of amplifiers were :

Noise level (root mean square),  $4\mu\text{V}$ .  
 Equivalent number ion pairs at input,  $\sim 1000$ .  
 Time constant of differentiating stage,  $200\ \mu\text{sec}$ .  
 Pulse growth time of amplifier,  $5\ \mu\text{sec}$ .

The amplifier was followed by a discriminator, a scale-of-100 counting unit and a cathode ray oscilloscope for visual monitoring of the amplifier output. The introduction of the neutron source increased the noise level by roughly a factor 2. The target discs were made circular with a slightly concave surface to counteract surface tensional forces which tended to cause a heavier deposit around the circumference of the discs. The reverse side of the target was flat and rested in a recess bored in a block of polythene.

The lithium acetate samples were converted into sulphate and a solution of approximately 5 mg. of the latter was evaporated on the circular target disc of 1 in. diam. All specimens were measured several times in alternation with blanks, with natural lithium sulphate samples and with no source present; corrections were made for background, contamination of the sample by natural radioactive substances, differences in weight, etc.

### Results and Discussion

In the most successful run, the front boundary of the "total lithium" was exceedingly sharp. Seven drops after the first spectroscopically noticeable trace of Li the concentration had risen to 88 % of the final value.

The result of the isotopic analysis is shown in Table II.

TABLE II  
DATA FROM THE CHROMATOGRAPHIC SEPARATION OF LITHIUM ISOTOPES

Sample No.	$v-162$	$C$	$\frac{{}^6\text{Li}}{{}^6\text{Li} + {}^7\text{Li}} = \frac{c_1}{C}$	Milli-equivalent ${}^7\text{Li}$ enriched
	$< 0$	Spectr. pure		
1	0-0.33	0.12	0.005	0.04
2	0.33-0.67	0.59	0.014	0.16
3	0.67-1.00	0.88	0.050	0.10
4	1.00-1.33	0.92	0.067	0.03
5	1.33-1.67	0.96	$0.075 = c/C$	Est. 0.01
6	1.67-2.00	1.0		Total 0.34

In Table II Col. 2 gives the volume of eluate after the lithium threshold (162 ml.), Col. 3 the concentration  $C$  of total lithium in the eluate sample, Col. 4 the ratio  ${}^6\text{Li}/({}^7\text{Li} + {}^6\text{Li})$  analyzed by the described nuclear reaction method, and Col. 5 the amount  $\Delta\mu_1$  of less adsorbed isotope ( ${}^7\text{Li}$ ) by which the frontal region has been enriched. This is given by

$$\Delta\mu_1 = \Delta v (c_1 - c_1^0 c_2/c_2^0) = \Delta v C (c_2^0 - c_2)/c_2^0.$$

These data disagree considerably with what one would expect from the theory.

First of all, the total amount  $\mu_1$  of  ${}^7\text{Li}$  separated is far too small. One would expect this to be

$$\mu_1 = (v - \alpha\bar{x}) \cdot c_1^0 (K - 1)/K, \text{ (see } ^2 5)$$

i.e., 2.8 milli-equivalents instead of 0.34 found.

Secondly, the gradient of the eluted boundary (at the centre, where  $c_2 = c_2^0/2$ ) is acc. to eqn. (5),

$$-\left[\frac{\partial c_2}{\partial x}\right]_{c_2^0/2} = \frac{(K-1)}{\Delta} \cdot \frac{c_2(c_2^0 - c_2)}{C} = \frac{(K-1) \cdot (c_2^0)^2}{4\Delta}.$$

Using  $K = 1.022$  (see <sup>18</sup>),  $c_2^0 = 0.075$  and the experimentally found  $(\partial c_2/\partial x)_v = 0.30$  then  $\Delta = 1.0 \times 10^{-4}$  g. (or  $2 \times 10^{-4}$  cm. length).

An estimate for the value of  $\Delta = (K_1 \text{ or } K_2) + K_3 + K_4$  using the values

$$\begin{aligned}\alpha &= 0.3 \text{ ml./g.}, \\ D_l &= 5 \times 10^{-6} \text{ cm.}^2/\text{sec.}, \\ D_s &= 10^{-6} \text{ cm.}^2/\text{sec.},\end{aligned}$$

leads to

$$\begin{aligned}K_1 &= 4 \times 10^{-4} \text{ g.} & K_2 &= 7 \times 10^{-4} \text{ g.} \\ K_3 &= 3 \times 10^{-6} \text{ g.} & K_4 &= < 10^{-6} \text{ g.} \\ \Delta &\simeq 7 \times 10^{-4} \text{ g.}\end{aligned}$$

It would appear from this that the assumptions made for  $K_1$  and  $K_2$  were too conservative. But it would be premature to draw conclusions until further experiments have been made. A similar reserve must also apply to remarks on the deficiency of <sup>7</sup>Li at the enriched front.

One might suspect that this result is due to a small proportion of an irreversible adsorption or exchange taking place at the front boundary, when the Li ions first contact the H<sup>+</sup> exchanger. In this way a small proportion of the enriched front would constantly be stripped off. Some support for this assumption may be gained from the (single) observation that no enrichment of <sup>6</sup>Li was observed at the rear of the Li band when the latter was eluted with HCl. If anything, a slight deficiency of <sup>6</sup>Li was observed, which would be the case if irreversibly adsorbed matter from the frontal region were eventually released by the stronger adsorbed H<sup>+</sup> ions.

It may be of interest to recall the early experiments of Taylor and Urey <sup>18</sup> on the chromatographic separation of the lithium isotopes in columns up to 30 m. in length. The large grain size ( $\sim 0.05$  cm.) and flow-rate ( $\sim 0.05$  ml./sec.) used precluded any spectacular enrichments, as, in spite of its length, the column cannot have possessed more than 1000-2000 "theoretical plates." But both the enrichment obtained and the actual amount of enriched material fell far short of what one would calculate from this figure and from the value of  $K = 1.022$ . In the present experiment, too, the amount of isotope separated was by one order too small.

There is an alternative to the irreversible adsorption hypothesis which also might explain the failure of these separations. It might be that the separation factor of the Li isotopes on a Li<sup>+</sup> saturated exchanger might be ten times smaller than the separation factor on an exchanger which is substantially in the Na<sup>+</sup> or H<sup>+</sup> form. It is the former which is effective in the chromatographic separations as carried out, but it is the latter which was independently determined by Taylor and Urey.

Some preliminary experiments showed that such factors can be operative. Thus a mixture of CuSO<sub>4</sub> and MnSO<sub>4</sub> did not separate when passed through a column of Zeo-Karb H.I., but gave some separation when 10 % of H<sub>2</sub>SO<sub>4</sub> was added to the mixture. Experiments to study these phenomena are planned.

<sup>18</sup> Taylor and Urey, *J. Chem. Physics*, 1938, **6**, 429.

### D. Separation of Neon Isotopes by Chromatographic Separation on Charcoal at $-196^{\circ}\text{C}$

**Experimental.**—The essential part of the apparatus is shown in Fig. 3 (a), (b). It consisted of a tube filled with charcoal, into one end of which neon of atmospheric pressure was fed. The other end led to a distributing system supplying a number of sample tubes which were analyzed with a mass spectrograph. The small dead space in the capillary leading from the charcoal to the stopcock was further reduced by filling it with an almost fitting glass rod. The charcoal column was filled with nitrogen and left in communication with a container of atmospheric neon. A Dewar vessel with liquid nitrogen was then slowly raised (by a motor), thus progressively cooling the charcoal tube from the rear end. This caused a constant inflow of neon into the charcoal up to the end of the low-temperature region, and an adsorption boundary forms which in every respect can be compared with a chromatographic boundary. The small amount of nitrogen originally added is more strongly adsorbed and disappears progressively as the charcoal tube is immersed. When the liquid  $\text{N}_2$  level had reached the top of the charcoal tube samples were taken.

A number of experiments were made varying the experimental conditions as described in Table III. Run 6 and the preliminary runs were made with a column shown in Fig. 3 (a), while subsequent experiments were made on the type of Fig. 3 (b), using thinner and longer tubes. In run 10 and 11 a different technique was used. The charcoal column was immersed from the start in liquid nitrogen and then filled with helium. Then through the column was passed a stream of neon which replaces the less adsorbed helium, and advances with a strongly self-sharpening front boundary. The arrival of the neon at the end of the charcoal column was noticed by the colour of a high-frequency discharge, and the front sample of the neon band was analyzed.

TABLE III  
EXPERIMENTS ON NEON ISOTOPE SEPARATION

No.	$\bar{x}$	$L$	$\varphi$ diam. of column	$d$ diam. of grains	$V_t$	Time of experiment $t$	Sample 1		Sample 2		Sample 3	
							$v - V_t$ ml.	$c_2^{\circ} - c_2$ $c_2^{\circ}$ $\times 100$	$v - V_t$ ml.	$c_2^{\circ} - c_2$ $c_2^{\circ}$ $\times 100$	$v - V_t$ ml.	$c_2^{\circ} - c_2$ $c_2^{\circ}$ $\times 100$
6	4.5	25	0.7	0.012	234	840	0.04	5.9	0.4-1.0	4.8	1.6-2.2	4.1
7	3.0	52	0.3	"	156	1320	lost		0.3-0.6	6.9	Sample 4 of No. 6 3.2-3.8      3.9	
8	"	"	"	"	"	"	0.2	7.1	2.0-2.1	5.0		
9	3.4	47	"	0.017	197	1200	0.03	8.4	0.3-0.6	8.0		
10	"	"	"	"	"	780	0.05	14.9				
11	"	"	"	"	"	4900	0.06	5.9				

### Discussion

The combined data of run 7, 8 and 9 render possible a calculation of  $(K - 1)$  by means of eqn. (11) and (12). With a lesser accuracy (on account of the scatter of the points) the data of run 6 can also be used. From run 7, 8 and 9 we obtain  $K = 1.0021$ ; from run 6,  $K = 1.0019$ .

Using the value of  $K = 1.0021$  we can calculate by means of eqn. (13) the values of  $\Delta$  for the various runs from the change observed in the isotopic ratio and obtain the figures in Col. 2 of Table IV.  $\Delta = K_2$  can also be calculated from eqn. (3b). (As mentioned before, in gas chromatography

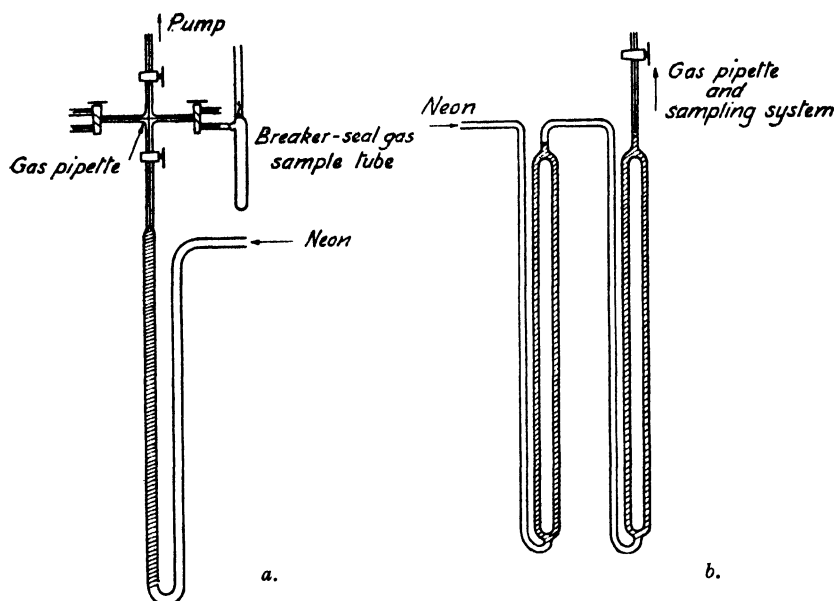


FIG. 3.—(a) Single charcoal tube, int. diam. 0.7 cm., length  $\sim 25$  cm.; (b) two double charcoal columns, each 25 cm. in length, about 0.3 cm. int. diam., for separation of neon isotopes at liquid nitrogen temperature.

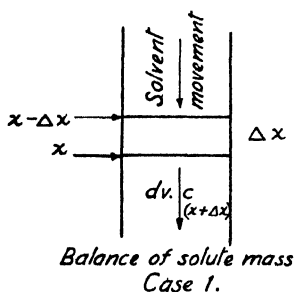
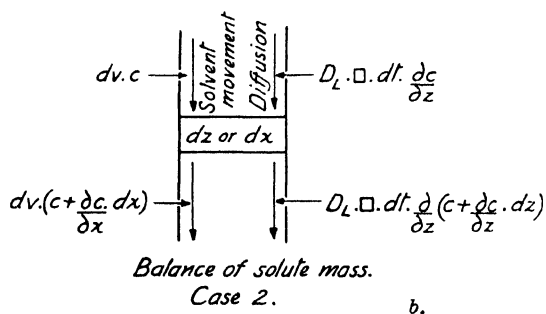


FIG. 4.—Scheme for balance of solute mass: (a) In case (i) (disturbance caused by finite grain size); (b) in case (ii) (disturbance caused by longitudinal diffusion).

longitudinal diffusion is the dominant disturbance.) The values used (at 77° K) are

$$A = \frac{\bar{x}}{L}, \quad F = \frac{V_i}{t} \cdot \frac{77}{293}, \quad D_{\text{Ne}} = 0.445 \times \frac{273 + 252}{77 + 252} \cdot \left(\frac{77}{273}\right)^{5/2} = 0.0298$$

(the Sutherland constant for neon being 252°).

TABLE IV

"THEORETICAL PLATE" VALUES CALCULATED FROM THE OBSERVATIONS AND FROM EQN. (3b)

No.	$10^3 \Delta$ calc. from observations with eqn. (13)	$10^3 \Delta/K_2$ calc. from eqn. (3b)	$\Delta/K_2$
6	6.7, 9.4, 10.8, 8.5; av. 8.8	7.4 (3.9)	1.2
7	2.8	1.8 (0.95)	1.5
8	2.0, 3.2; av. 2.6	1.8 (0.95)	1.4
9	2.4, 2.4	2.0 (1.05)	1.2
10	0.64	1.3 (0.68)	0.49
11	4.9	8.3 (4.4)	0.59

It can be noted that the variations of flow-rate and diameter of the tube are well represented by eqn. (3b) for both types of experiment. Also, no effect of the change of grain size is observable, as would be expected for a diffusion-controlled disturbance. As in the case of the Li separation, the best experimental set-up (10 and 11) gives a boundary which is sharper (i.e.,  $\Delta$  is smaller) than expected, though the discrepancy is only by a factor of 1.8. The reason for this is probably that only a fraction of the pore space  $\alpha$  is used in the longitudinal transport of the gas and consequently the linear flow-rate is faster than anticipated. If this is confirmed by further experiments it will be proposed to change the  $\sqrt{2}$  in eqn. (3b) against an empirical factor around 2.7. The bracketed figures in Col. 3 of Table IV correspond to this change.

No reason has so far been found why the first set of runs, using the movement of the liquid nitrogen bath as the speed-determining factor, should give a less favourable separation (i.e., a larger  $\Delta/K_2$ ). But the absence of any influence of the tube diameter and of the time seems to indicate that this is not due to any lack of temperature equilibrium.

In conclusion, it may be said that the experiments support the theoretical deductions as well as may be expected, so that some reliance may be placed on predictions made on this basis. This enables one to draw some conclusions concerning the value of the chromatographic technique for the purpose of isotope separation. It is quite apparent that it cannot be easily adapted to isotope separation in bulk, when the separation factor is small. Both in the case of Li and of neon the amounts of enriched isotope have been of the order of a milligram, and while the scaling-up to gram quantities is conceivable, the difficulties of maintaining sharp boundaries under such conditions should not be underestimated. There is only one field in which the chromatographic method is likely to be superior and this applies to cases where the absolute quantities involved are exceedingly small and where the separation factor is comparatively large. Such a case, for example, may be the separation of  $^3\text{He}$  from enriched helium. With a theoretical plate height of 0.2 mm. which has been achieved in the neon experiments and a separation factor which, at the temperature of liquid hydrogen, should not be less than  $K = 1.1$ , it ought to be possible to enrich  $^3\text{He}$  by a factor of 10 in a column of 1 m. length.

## Appendix

### Derivation of the Constants of the Disturbance Term

#### CASE (I) (FINITE GRAIN SIZE)

We consider (see Fig. 4 (a)) the balance of solute in a small section  $\Delta x$  of the column, which corresponds to a "theoretical plate."  $\Delta x$  may be assumed to be identical with the grain diameter  $d$ . The content of the section changes from

$$\Delta x f(c) \text{ to } \Delta x \left( f(c) + \frac{\partial f(c)}{\partial v} \cdot dv \right).$$

Total balance of mass requires that

$$\left( \frac{\partial f(c)}{\partial v} \right)_x dv \Delta x + dv(c_x) - c_{(x-\Delta x)} = 0. \quad (14)$$

By means of a Taylor expansion we can write

$$c_{(x-\Delta x)} = c_x - \Delta x \cdot \frac{\partial c}{\partial x} + \frac{1}{2} (\Delta x)^2 \cdot \frac{\partial^2 c}{\partial x^2} \dots \quad (15)$$

which leads to

$$\left( \frac{\partial f(c)}{\partial v} \right)_x + \left( \frac{\partial c}{\partial x} \right)_v - \frac{\Delta x}{2} \cdot \left( \frac{\partial^2 c}{\partial x^2} \right)_v = 0. \quad (16)$$

From this is obtained, for the final state of a self-sharpening boundary, the concentration gradient in the effluent as given by eqn. (3), (3a).

#### CASE (II) (DIFFUSION)

We consider the balance of solute in a small section of the column where the content of the section changes from

$$dx \cdot f(c) \text{ to } dx \left( f(c) + \frac{\partial f(c)}{\partial v} \cdot dv \right).$$

The balance from solvent and diffusion movements plus change of content of section (see Fig. 4 (b)) equals zero; thus:

$$\frac{\partial c}{\partial x} dv \cdot dx - D_t \square dz \cdot dt \cdot \frac{\partial^2 c}{\partial z^2} + \frac{\partial f(c)}{\partial v} \cdot dv \cdot dx = 0. \quad (17)$$

We replace

$$\begin{aligned} dt &\text{ by } dv/F, \\ dz &\text{ by } dx/A, \\ \square &\text{ by } A \cdot \alpha/\sqrt{2}, \end{aligned}$$

(the  $\sqrt{2}$  should account for the increased length of the diffusion path between the grains) and obtain

$$\left( \frac{\partial f(c)}{\partial v} \right)_x + \left( \frac{\partial c}{\partial x} \right)_v - \frac{D_t \alpha A^2}{F} \cdot \left( \frac{\partial^2 c}{\partial x^2} \right)_v = 0. \quad (18)$$

For the final state of a self-sharpening boundary this leads to eqn. (3), (3b).

#### CASE (III) (NON-EQUILIBRIUM DUE TO GRAIN DIFFUSION)

Mass conservation requires that

$$\left( \frac{\partial c}{\partial x} \right)_v + \left( \frac{\partial(q^* + \alpha c)}{\partial v} \right)_x = 0. \quad (19)$$

This is eqn. (17) without the diffusion term, with the equilibrium term replaced by  $q^* + \alpha c$ . The change of absorbate at a given point can be represented with good approximation by

$$\frac{\partial q^*}{\partial t} = \frac{D_s}{ad^2} (f^*(c) - q^*), \quad (20)$$

which means that the rate of diffusion into the grains is essentially proportional to the amount still required to produce equilibrium. Replacing  $t$  by  $v/F$ ,

$$\frac{\partial q^*}{\partial v} = \frac{D_s}{ad^2 F} (f^*(c) - q^*), \quad (21)$$

and differentiating by  $\partial v$

$$-\frac{\alpha d^2 F}{D_s} \cdot \frac{\partial^2 q^*}{\partial v^2} = \frac{\partial f^*(c)}{\partial v} - \frac{\partial q^*}{\partial v}.$$

Elimination of  $\partial q^*/\partial v$  in eqn. (19) then results in

$$\frac{\partial c}{\partial x} + \frac{\partial f(c)}{\partial v} - \frac{\alpha d^2 F}{D_s} \cdot \frac{\partial^2 q^*}{\partial v^2} = 0. \quad (22)$$

Differentiation of (19) by  $\partial v$  gives

$$\frac{\partial}{\partial v} \left( \frac{\partial(q^* + \alpha c)}{\partial v} \right) = -\frac{\partial}{\partial v} \left( \frac{\partial c}{\partial x} \right) \simeq \frac{\partial^2 q^*}{\partial v^2}, \quad (23)$$

so that (22) can be written :

$$\left( \frac{\partial f(c)}{\partial v} \right)_x + \left( \frac{\partial c}{\partial x} \right)_v + \frac{\alpha d^2 F}{D_s} \cdot \frac{\partial^2 c}{\partial x \cdot \partial v} = 0. \quad (24)$$

For a front boundary which moves without change of form,

$$\left( \frac{\partial v}{\partial x} \right)_c - \alpha = \frac{dq^*}{dc} = \frac{f^*(c^0)}{c^0}.$$

As  $q^* = 0$  for  $c = 0$ , we obtain

$$q^* = c \cdot \frac{f^*(c^0)}{c^0}. \quad (25)$$

This permits the integration of eqn. (24) leading to eqn. (3), (3c).

#### CASE (IV) (NON-EQUILIBRIUM DUE TO DIFFUSION THROUGH A THIN LIQUID FILM SURROUNDING THE GRAIN)

The balance of solute mass again leads to eqn. (19). The change of  $q^*$  by diffusion through a layer of thickness  $\delta$  cm. during the time  $dt = dv/F$  for 1 g. sorbent, the surface  $O$  of which is

$$O = \frac{\pi d^2}{1/6\pi d^3 s} = \frac{6}{ds} \quad (26)$$

is approximately given by

$$dq^* = \frac{D_l O (c - c_s) \cdot dt}{\delta} = \frac{6 D_l (c - c_s) dt}{\delta ds F}, \quad (27)$$

where  $c_s$  is the concentration on the sorbent side of the liquid film, in equilibrium with  $q^*$ .

Thus

$$c_s = \varphi(q^*).$$

The change of concentration at the point  $x$  is therefore given by

$$\left( \frac{\partial q^*}{\partial v} \right)_x = \frac{6 D_l}{\delta ds \cdot F} (c - \varphi(q^*)). \quad (28)$$

As in the final state of the self-sharpening boundary  $q^*$  can be replaced by  $\frac{c}{c^0} \cdot f(c^0)$  (see eqn. (25)) ; this leads to eqn. (4), (4a).

The experiments with lithium isotopes were carried out for the Department of Atomic Energy, M.O.S., at the University Science Laboratories at Durham (E. G.). The method for isotopic analysis of the lithium was developed and the measurements done in the laboratory of Dr. B. Rotblat, Physics Department, Liverpool University (K. H. B.). The work with neon isotopes was done at the Atomic Energy Research Establishment, Harwell (E. G. and G. P. K.). Our thanks are due to the Director, A.E.R.E., for permission to publish this paper, to the Durham Colleges and Prof. F. A. Paneth for laboratory facilities and to Dr. B. Rotblat (University of Liverpool), Dr. H. London (A.E.R.E.) and Dr. B. Davison (A.E.R.E.) for valuable discussions.

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# LARGE-SCALE SEPARATION OF RARE-EARTH SALTS AND THE PREPARATION OF THE PURE METALS

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For many years one of the most difficult processes in the field of chemistry has been the separation of the rare earths from each other into their pure states. Their chemical and physical properties are so similar that in general a single operation leads only to a partial separation or enrichment.

Ever since the beginning of the Manhattan Project there has been a constant demand for samples of rare earths of exceptional purity in gram amounts or greater. This demand arose for numerous reasons, but mainly because some of the rare earths are formed as fission fragments during fission of the heavy elements. It was highly desirable, therefore, to have a means of preparing pure rare earths so that their nuclear properties could be studied and also to allow a more thorough consideration of their chemical behaviour.

In general, the best means of separating these elements has been the well-known, but laborious, method of fractional crystallization, etc., as used by James, Hopkins and others. Cerium with its quadrivalent state and samarium, europium and ytterbium with their divalent states which do permit a means of separation from the normal trivalent rare earth ions are exceptions.

Starting in 1945, the Ames Laboratory of the United States Atomic Energy Commission developed a series of ion-exchange processes for separating adjacent rare earths on the macro scale. These all involved the adsorption of the mixed rare-earth ions on the top of resinous ion-exchange columns such as Amberlite IR-100 and the later elution of these rare earths down the columns by means of citrate solutions of various concentrations and pH values. The conditions for the good separation of the mixed rare-earth into pure rare-earth components are discussed in detail in the paper.

The Ames Laboratory has also developed processes for producing pure rare-earth metals. Lanthanum, cerium, praseodymium, neodymium and yttrium have been prepared as massive metal to date.

The group of elements known as the rare earths and the similar elements scandium, yttrium and lanthanum usually associated with them have fascinated me ever since my graduate school-days. I believe that a study of these elements offers one of the best keys to a better understanding of the relationships between atomic structure and the various physical and chemical properties which metals and compounds possess. The rare earths are a group of some 14 elements which start with atomic number 58 and extend through atomic number 71. They have almost identical physical and chemical properties. These properties are so similar to those of lanthanum, element 57, that in the condensed periodic table these elements are always grouped together in the square with lanthanum. Almost all the physical and chemical properties of a substance are primarily determined by the outermost electrons of the atoms of the substance since it is these outermost electrons which are involved in chemical bonds and which are shifted about when chemical processes take place. In this group of elements there are three electrons which principally determine their chemical behaviour so that they all have a valency of 3 and, therefore, form very similar compounds which are extremely difficult to separate from one another. The electronic structures of the elements as they exist in solid salts are shown in Table I. Starting with cerium, an incomplete inner shell, the  $4f$  begins to take up electrons and since this inner sub-shell is protected by the completed  $5s5p$  sub-shells which project farther out from the centre of the atom, these inner

electrons are very little affected by the bonding with the neighbouring atoms. These inner electrons are, therefore, frequently unpaired electrons and give rise to the paramagnetic properties of the rare-earth salts. Even in the solid state, these inner electrons are so well protected from external bonding and coupling by the completed sub-shells outside them that, as a first crude approximation, the energy states arising from them can be treated theoretically<sup>1</sup> in the same manner as if the rare-earth atoms were in a gaseous form. In a more precise calculation, however, we have to take into consideration the fact that these states are affected by the surrounding atoms in the solid since these atoms subject the rare-earth atom to strong electric fields. It has been found that if the solid salts are cooled to low temperatures, the transitions between energy states arising from the inner electrons give rise to extremely sharp absorption spectra and that the fine structure of these absorption spectra varies from compound to compound.

TABLE I  
ELECTRONIC ARRANGEMENTS OF THE RARE-EARTH ELEMENTS IN THEIR SALTS

	At. No.	1 <sub>1</sub>	2 <sub>1</sub> 2 <sub>2</sub>	3 <sub>1</sub> 3 <sub>2</sub> 3 <sub>3</sub>	4 <sub>1</sub> 4 <sub>2</sub> 4 <sub>3</sub> 4 <sub>4</sub>	5 <sub>1</sub> 5 <sub>2</sub> 5 <sub>3</sub> 5 <sub>4</sub> 5 <sub>5</sub>	6 <sub>1</sub>
Sc	21	2	2 6	2 6 1	2		
Y	39	2	2 6	2 6 10	2 6 1 0	2	
Ba	56	2	2 6	2 6 10	2 6 10 0	2 6 0 0 0	(2)
La	57	2	2 6	2 6 10	2 6 10 0	2 6 (1) 0 0	(2)
Ce	58	2	2 6	2 6 10	2 6 10 1	2 6 (1) 0 0	(2)
Pr	59	2	2 6	2 6 10	2 6 10 2	2 6 (1) 0 0	(2)
Nd	60	2	2 6	2 6 10	2 6 10 3	2 6 (1) 0 0	(2)
61	61	2	2 6	2 6 10	2 6 10 4	2 6 (1) 0 0	(2)
Sm	62	2	2 6	2 6 10	2 6 10 5	2 6 (1) 0 0	(2)
Eu	63	2	2 6	2 6 10	2 6 10 6	2 6 (1) 0 0	(2)
Gd	64	2	2 6	2 6 10	2 6 10 7	2 6 (1) 0 0	(2)
Tb	65	2	2 6	2 6 10	2 6 10 8	2 6 (1) 0 0	(2)
Dy	66	2	2 6	2 6 10	2 6 10 9	2 6 (1) 0 0	(2)
Ho	67	2	2 6	2 6 10	2 6 10 10	2 6 (1) 0 0	(2)
Er	68	2	2 6	2 6 10	2 6 10 11	2 6 (1) 0 0	(2)
Tm	69	2	2 6	2 6 10	2 6 10 12	2 6 (1) 0 0	(2)
Yb	70	2	2 6	2 6 10	2 6 10 13	2 6 (1) 0 0	(2)
Lu	71	2	2 6	2 6 10	2 6 10 14	2 6 (1) 0 0	(2)
Hf	72	2	2 6	2 6 10	2 6 10 14	2 6 (2) 0 0	(2)

( ) Involved in valency bonds.

Using quantum mechanics it is possible to calculate theoretically where these sharp lines should appear in the spectra and how the groupings of the fine structure should vary as the symmetry and strength of the fields vary due to the surrounding atoms. We have, therefore, within this group of elements, a chance to study directly the short-range forces which surround an atom in a solid and to correlate the many physical and chemical properties of the solid with the absorption spectra. This then enables us to correlate the properties of the solid directly with such fundamental data as the charge on the electron, the energy states of the atoms and the distances and positions of the surrounding atoms in the solid. Further, since the chemical and physical properties are primarily determined by the three outermost electrons these elements form a series of very similar compounds having the same valency forces and, therefore, similar crystal structures. However, since the charge on the nucleus increases as the atomic number

<sup>1</sup> Hund, *Linienpektren und periodisches System der Elemente* (J. Springer, Berlin, 1927).

increases, the inner shells are pulled in closer so that the lattice parameters change in a predictable way. Occasionally in these series, when this shrinking has reached the point where the atoms can no longer be grouped together without strain due to their atomic radii, there will result a change in the crystal structure which should also be predictable. Since these factors can be taken into account in almost any theory put forward concerning the structure of solids or metals, or the solubility of salts, etc., the compounds of these elements, their alloys or even the pure metals themselves, offer the ideal group for checking the present theories concerning the solid or liquid state and the various theories of solution. It should be pointed out that most of these theories contain a number of arbitrary constants. If an attempt is made to check these theories using other elements in the periodic table, so many factors vary from element to element that it is almost always possible to find arbitrary constants which will fit. However, in the rare-earth group it is possible to hold many of these variables constant, and the variables that do change should do so in a predictable manner. Accordingly, in any theory where the constants are made to fit one member of the rare-earth series, they should, after suitable corrections have been made, fit all of the members. If they do not, something must be wrong with the theory. Once the theories have been made to fit the entire rare-earth group, then it should be possible to deal separately with the changes which will take place when the valencies and crystal structures change in going from one element to another in various parts of the periodic table.

It should be pointed out that when a sub-group is completed or half-completed a very stable configuration is formed. In these cases the energy required to remove an electron from the  $4f$  shell or from one of the outer valency shells should not differ greatly. This accounts for the fact that certain of the rare-earth elements can exist in other valency states than the trivalent state. For example, in cerium the single  $4f$  electron can go to the outside and give rise to a 4-valent cerium. There is a slight tendency for the same thing to happen with terbium where the half-completed shell of 7 tends to permit the 8th electron to go to the outside. Terbium in its oxide form is known to possess a higher valency state, but in solution no higher valency state has been reported. It appears that the stabilizing action of the half-shell is not sufficient to make the higher state stable in the presence of water. On the other hand, when one more electron is needed to complete or half-complete the shell, an outer electron can go to the inside, thus giving rise to the divalent state, as it does in europium and ytterbium and to some extent in samarium. In general, these anomalous valency states are less stable than the trivalent state, but in separating these particular rare earths from the others advantage can be taken of the fact that such valencies do exist. Considerable success has been obtained, in this regard, by workers such as Marsh,<sup>2</sup> Hopkins,<sup>3</sup> Yntema,<sup>4</sup> McCoy,<sup>5</sup> etc.

For many years one of the most difficult processes in the field of chemistry has been the separation of rare-earth mixtures into their pure salts. The chemical and physical properties, aside from the exceptions mentioned

<sup>2</sup> Marsh, *J. Chem. Soc.*, 1937, 1367; 1942, 398; 1942, 523; 1943, 8; 1943, 531.

<sup>3</sup> Andrieth, Jukkola, Meints and Hopkins, *J. Amer. Chem. Soc.*, 1931, **53**, 1805. Meints, Hopkins and Andrieth, *Z. anorg. Chem.*, 1933, **211**, 237. Jukkola, Andrieth and Hopkins, *J. Amer. Chem. Soc.*, 1934, **56**, 303. West and Hopkins, *J. Amer. Chem. Soc.*, 1935, **57**, 2185. Pearce, Naeser and Hopkins, *Trans. Electrochem. Soc.*, 1936, **69**, 8.

<sup>4</sup> Yntema, *J. Amer. Chem. Soc.*, 1930, **52**, 2782. Yntema and Ball, *J. Amer. Chem. Soc.*, 1930, **52**, 4264.

<sup>5</sup> McCoy, *J. Amer. Chem. Soc.*, 1935, **57**, 1756; 1936, **58**, 1577; 1936, **58**, 2279; 1937, **59**, 1131; 1941, **63**, 1622; 1941, **63**, 3432. McCoy and Hammond, *J. Amer. Chem. Soc.*, 1942, **64**, 1009.

above, are so similar that usually a single operation leads only to a partial separation or enrichment. In the past, the best means of separating these elements have been the well-known but laborious methods of fractional crystallization, fractional decomposition, etc., as developed by workers such as Hopkins,<sup>6</sup> James,<sup>7</sup> Urbain<sup>8</sup> and many others. These processes were tedious and required a great deal of drive, patience and determination on the part of the research workers in order to obtain small amounts of the pure rare earths. The same operations had to be repeated many times and in some of the rarer and more difficult to separate rare earths it has required up to 20,000 operations to accomplish purification. Therefore, except to the few stout souls who were willing to devote a lifetime to this sort of study, the rare earths were not generally available. While these pioneers were very generous in lending samples to other workers, the quantities they had were strictly limited. Also, it is understandable that when these scientists had devoted many years to obtaining a few grams of the rare earths they themselves wanted to study the properties of the salts. For these reasons the rare earths have not been nearly as extensively studied as the other elements of the periodic table and many of their properties, particularly the properties of the metals and their alloys, are almost unknown even to the present day.

Ever since the beginning of the Manhattan Project there has been a constant demand for samples of rare earths of exceptional purity in gram amounts or greater. This demand arose for numerous reasons, but mainly because some of the rare earths are formed as fission fragments during the fission of the heavy elements. In other words, they are among the ashes of the atomic reaction. It was highly desirable, therefore, to have a more satisfactory means of preparing pure rare earths so that their nuclear properties could be studied, and also to allow a more thorough determination of their chemical behaviour.

A number of workers have reported, in the literature, studies on the application of chromatographic and ion-exchange methods to the separation of rare earths. While they obtained some enrichment, their results were not sufficiently promising to lead to further intensive investigations or to the production of pure rare earths in quantity. Shortly after the Plutonium Project was organized at Chicago, Dr. George Boyd<sup>9</sup> introduced the use of Amberlite as a research tool in studying the complicated mixtures which occur when uranium fissions. Dr. Tompkins, Dr. Khym and Dr. Cohn<sup>10</sup> used Amberlite rather extensively in their studies of fission products. At that time all sections of the Manhattan Project worked closely together and at a meeting of section chiefs in December, 1944, Dr. Cohn stated that they had succeeded fairly well in separating the cerium fission fragments from the yttrium fission fragments. They accomplished this by using citric acid solutions of controlled pH for eluting the various ions from Amberlite columns upon which the fission fragment ions had been adsorbed. He also mentioned that in the eluant between the rich yttrium fraction and the cerium fraction there was a concentration of the other rare earths. It

<sup>6</sup> Hopkins, numerous publications in the literature.

<sup>7</sup> James, numerous publications in the literature.

<sup>8</sup> Urbain, numerous publications in the literature.

<sup>9</sup> Boyd, Schubert and Adamson, *J. Amer. Chem. Soc.*, 1947, **69**, 2818. Boyd, Adamson and Myers, *J. Amer. Chem. Soc.*, 1947, **69**, 2836. Boyd, Myers and Adamson, *J. Amer. Chem. Soc.*, 1947, **69**, 2849. Ketelle and Boyd, *J. Amer. Chem. Soc.*, 1947, **69**, 2800.

<sup>10</sup> Tompkins, Khym and Cohn, *J. Amer. Chem. Soc.*, 1947, **69**, 2769. Harris and Tompkins, *J. Amer. Chem. Soc.*, 1947, **69**, 2792. Tompkins and Mayer, *J. Amer. Chem. Soc.*, 1947, **69**, 2859.

occurred to the author that this offered a possibility of actually separating adjacent rare earths in macro quantities if the right conditions could be found. The Ames Laboratory set to work and during the next few months succeeded in separating gram quantities of praseodymium from neodymium. When this work was reported before the section chiefs' council in April, Dr. Hume<sup>11</sup> reported that the men at Oak Ridge had also succeeded in separating the rare earths on the micro and tracer scale. The history of how this development took place has been described in some detail in a paper by Johnson, Quill and Daniels,<sup>12</sup> so I shall not go into it further here, but shall confine the rest of my paper to the work at Ames where we continued to develop the macro separation of non-radioactive rare-earth atoms.

### Macro Separation of Non-radioactive Rare-earth Atoms

Since, at best, any single chemical operation gives only a slight enrichment of one adjacent rare earth over another, it is obvious that to separate successfully the rare earths into their pure fractions it will be necessary to have a process which automatically repeats the same operation many times. These conditions can

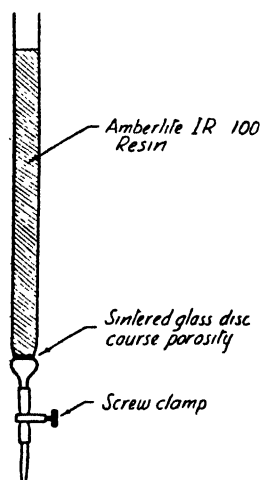


FIG. 1.—Schematic diagram of an Amberlite column with a sintered glass support.

be fulfilled in an ion-exchange column. In the work which we carried out at Ames we took advantage of the ion-exchange type column, using a synthetic resin either Amberlite IR-1 or -100 or the high-capacity resins such as the Dow Chemical Company and others put out. Most of our work was done with the Amberlite IR-100 which is a synthetic resin built up from a condensation of phenol, formaldehyde and sulphuric acid. The apparatus consisted essentially of a long tube into which the resin had been packed; the resin was supported at the bottom by a metal screen or a sintered glass plate and a suitable pinch clamp was located at the bottom so that the flow rate through the column could be controlled (Fig. 1). As will be shown later, the particle size can be very important in the separation, so generally the resin was classified either by sieving, or in the earlier stage by back-washing, so that the fine material was washed out the top and the coarser particles were segregated according to size down the column. The size of the column is immaterial since a column of any size will work providing certain conditions are fulfilled. The columns can vary all the way from capillary tubes a few centimetres in length, which might be used when tracer

activities are separated from each other, up to columns several feet in length and many inches in diameter. In most of our work for experimental purposes we use column beds from 30 to 120 cm. length and about 22 mm. diam. Once the Amberlite had been packed and classified in the column, it was put in the acid cycle by pouring a 5 % hydrochloric acid solution through the resin, thus putting hydrogen ions on the active exchange points of the resin. Next, a slightly acid solution of the mixed rare-earth chlorides was poured in the top of the column and allowed to adsorb in a band in the upper section. Usually the width of the adsorbed band of rare earths in the column occupied from one-tenth to one-third of the column length. The column was then washed out with distilled water to remove the chloride ions. It was then in condition to start a rare-earth separation run. Previously a solution of citric acid of fixed concentration had been prepared in a large reservoir and brought to the desired pH by adding ammonium hydroxide until a pH meter gave the selected reading.

<sup>11</sup> Swartout and Hume, *Metallurgical Projects Report*, CN-1839 (July 10, 1944).

<sup>12</sup> Johnson, Quill and Daniels, *A.C.S. Chem. Eng. News*, 1947, **25**, 2494.



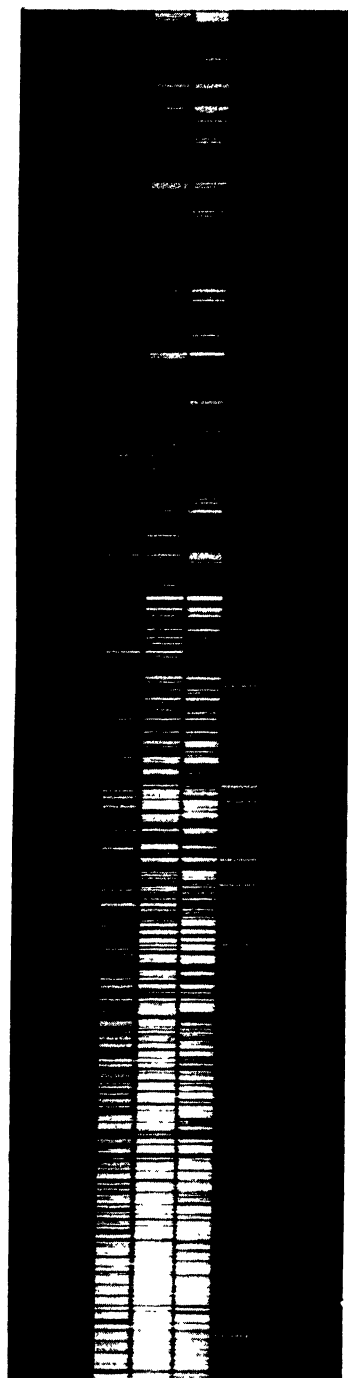


FIG. 2.—Typical second order rare-earth spectra from a 21-ft. grating spectrograph having a dispersion of  $2.5 \text{ \AA}^{\circ}/\text{mm}$ . exposures from top to bottom: pure neodymium, a mixture of neodymium and samarium, pure samarium and the relatively simple iron spectrum.

This solution was started flowing slowly through the column and samples were taken from the bottom periodically.

While the rare-earth ions are tightly adsorbed on the Amberlite in the low pH range, at the pH where the citric acid solution is flowing through the column an equilibrium is set up between the Amberlite-rare-earth complex and the citrate complex. The equilibrium constants differ slightly for the different rare earths and this causes the heavier rare earths to move down the column more rapidly than the lighter rare earths. Since the shifting back and forth between the resin and the complex ion must occur thousands of times as the rare earths move down the column, this ultimately results in the separation of the rare earths which come out at the bottom. The exact mechanism by which this occurs is very complex, so until we have more data we do not intend to say very much about the nature of the mechanism involved. Each time we complete a set of experiments, we believe we understand the mechanism and then invariably find that with further data this mechanism has to be modified in the light of the additional results. I think we can say, however, that there is no doubt that there is an equilibrium competition set up between the various ions and that in the pH range which we have so far investigated there are at least several rare-earth citrate complexes which are involved. We have spectrophotometric evidence from studying the adsorption lines of the neodymium ion and the neodymium complexes in solution that there are at least four citrate complexes involved in the equilibrium in the pH range studied. In addition, there is the possibility that the solubility of the hydrated salts, the formation of Werner-type<sup>18</sup> complexes, etc., may be involved in a complete explanation of all the phenomena encountered. Further, in some ranges the rate of establishing the complex equilibrium is the rate-determining steps but in others the diffusion rate in the liquid or the diffusion rate in the Amberlite may be the predominating rate-controlling step; we are convinced that the best way to attack this problem is to obtain adequate data and to leave the theoretical explanation until we know the facts. Also, we have been seriously handicapped in our work in that we have not had available a number of the rarer rare earths in pure form so that we could investigate their physical constants and the analytical procedures for determining them quantitatively. We have had to prepare these rare-earth salts the best we could by taking the richest fraction from the various runs. We then used these salts to determine the constants and procedures for subsequent runs. In determining such constants as extinction coefficients, and such optimum conditions as the best pH range, etc., we have had to, in a manner of speaking, lift ourselves by our boot straps. It is only recently that we have obtained some of the rarer rare earths in high concentrations in reasonable amounts. It should also be pointed out that the analytical methods for determining the impurities of the rare earths in each other are not at all satisfactory. The spectrophotometric methods are good for only the paramagnetic salts and at best can determine the concentrations to only 1%–2%. The emission spectra of the rare earths are so rich in lines that even in the second-order of a 21-ft. grating (Fig. 2) the lines are frequently broader than the distances between the lines. It is, therefore, difficult to obtain the purities spectrographically although it can be done and is being done in our laboratory. However, this method of analysis requires that calibration curves be run on the pure rare earths for the impurities of the other rare earths and if you do not have supplies of all the pure rare earths in sufficient quantity to set up these standards, the analyses may subsequently prove to be in error as purer sources of the major rare-earth constituents become available. I might say in passing that the so-called spectrographically pure rare earths obtained from commercial companies in four out of five cases turn out to be nowhere near as spectrographically pure as claimed. In one case we determined the extinction coefficient of an adsorption band from a so-called spectrographically pure standard. Later we found in using this value that our salt was analyzing 120% purity. The atomic-weight method of determining purities is not very sensitive since the atomic weights of all the

<sup>18</sup> Werner, *New Ideas on Inorganic Chemistry*, Tran. by Hedley (Longmans, Green & Co., London, 1911).



## SEPARATION OF RARE-EARTH SALTS

rare earths are so close together. Perhaps the most sensitive method of determining purity is to send a sample to one of the atomic reactors and have it bombarded with neutrons; the impurities will then give activities which can be followed by counters and some idea of the absolute purity can be determined. However, it is not usually convenient to send a sample to Oak Ridge for analysis and in most cases the spectrographic methods are sufficiently accurate to detect impurities which occur in significant amounts.

Fig. 3 shows the separations which can be obtained between neodymium and praseodymium using a 5 % citric acid solution at a pH of about 2.55. This represents the separation of a mixture of about equal amounts of praseodymium and neodymium on a 70-in. column operating at near optimum conditions. It will be noticed that about 30 % of the neodymium comes out spectrographically pure and that about 85 % or 90 % of it comes out better than 95 % pure. However, with such high concentrations of citric acid, the cost of this acid became very great when scaled up to large operations. It was very desirable to see whether a more dilute solution of citric acid could be used.

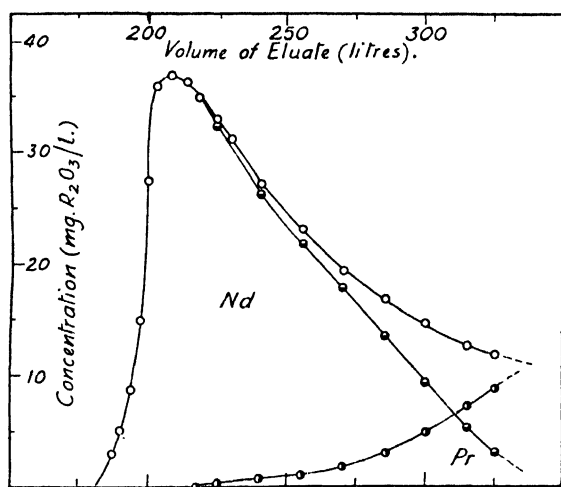


Fig. 3.—The elution of a sample, 0.25 g./sq. cm., analyzing 51.7 %  $\text{Pr}_6\text{O}_{11}$  and 48.3 %  $\text{Nd}_2\text{O}_3$ , from a bed of Amberlite IR-1 resin,  $6.4 \times 175$  cm., using 5.0 % citrate solution at a pH value of 2.55 and a flow-rate of 1.5 cm./min.

Fig. 4 shows one of our pilot plants for separating rare earths. Here we are using 8-ft. columns of 4 in. diam. and are adsorbing on top of each column from 50 to 100 g. mixed rare-earth oxides after they have been converted to the chlorides. The samples are collected in 45-l. bottles and these are changed every 12 hr. The citric acid elution solution is mixed in the large tank shown in the background. In our more recent work we are using the same columns with 4-ft. beds. At this point it should be emphasized that in obtaining rare earths on the macro scale the important thing is not how much rare earth a given adsorbent will hold or how pure an infinitesimal fraction can be obtained from the bottom of the columns, but rather how many grams of a rare-earth salt of a given purity can be obtained per man-month of labour involved. Therefore, since it takes very little more energy to watch 20 columns than it does to watch one, it pays to make the set-up on a multiple scale. When operating on such a large scale as this the cost of chemicals has to be seriously considered and if a more dilute solution can be made to work, it may be desirable to use the more dilute solution even though the separations are slightly inferior.

Table II shows the composition of the crude didymium which we adsorbed on the columns in our early production runs where we were separating praseodymium, neodymium and samarium. This material can be obtained from the Lindsay Light and Chemical Company and is the residue from the monazite



FIG. 4.—Pilot plant for the separation of rare earths by ion-exchange columns.

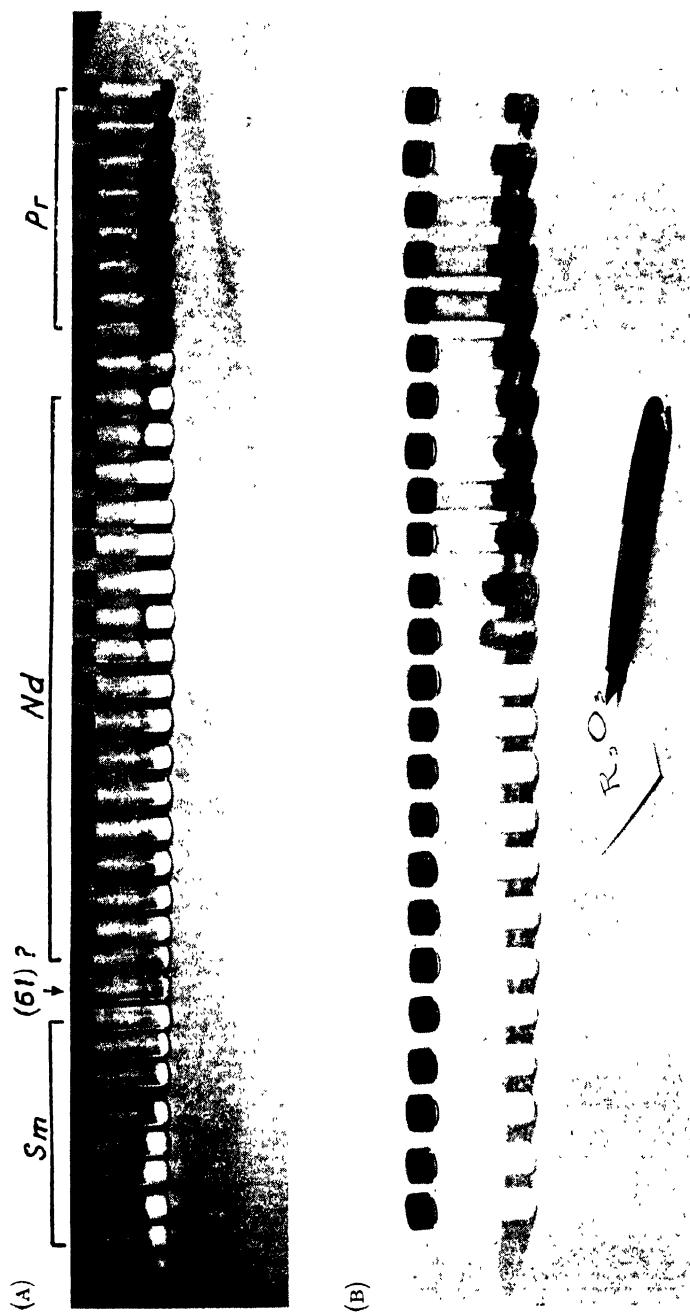


Fig. 5.—Typical separations of rare-earth elements by ion-exchange columns: upper series of vials (A) using 0.5 % citrate solution at a pH of 3.9; lower series (B) using 0.1 % citrate at a pH of 6.0.

sand after they have removed the thorium, cerium and lanthanum for which they have commercial markets.

Fig. 5 shows how well this mixture is separated by one pass through the column. The upper row of vials shows an early separation where 0.5 % citric acid at a pH of 3.9 was used. The lower row of vials represents a run using 0.1 % citric acid at a pH of 6.0. Each of the bottles which were taken off at 12-hr. intervals had its rare-earth fraction precipitated by adding oxalic acid. The insoluble oxalate was filtered out and a prorated fraction of the precipitate was collected in each of the little vials. Therefore, each vial can be considered a 12-hr. period. Of course, there was a considerable time element before any rare earth broke through the bottom of the column. The gadolinium and terbium fractions came off first, then the samarium fraction. The 61 fraction would have come off next if any appreciable amount had been present. Next came the neodymium fraction and finally the praseodymium fraction.

Fig. 6 shows the results of near optimum conditions for the separation of Lindsay's "neodymium carbonate" and is a graphical representation of Fig. 5 (A). It will be noticed that the quantities of pure salts which can be obtained using a pH of 3.9 and a citric acid concentration of one-tenth of 5 % are considerably better than those obtained when using a 5 %-citrate solution at a pH of 2.55.

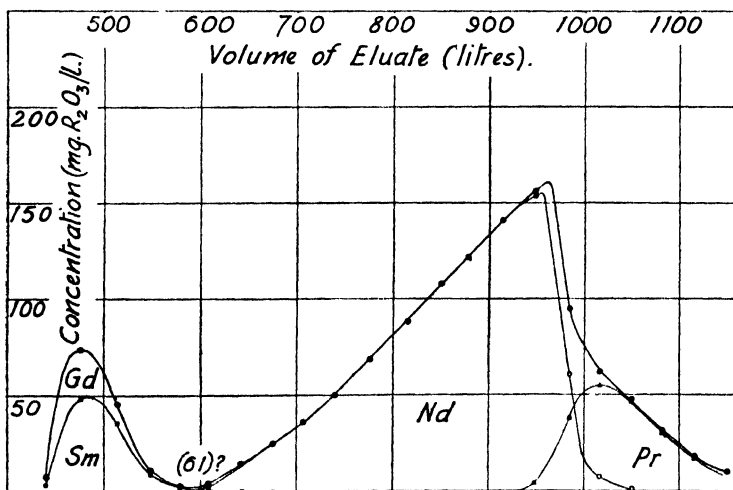


Fig. 6.—Large scale separation of a crude rare-earth mixture by ion exchange; typical elution curve obtained at a pH of 3.9 with 0.5 % citrate solution: ●, total  $R_2O_3$ ; □,  $Sm_2O_3$ ; ○,  $Nd_2O_3$ ; Δ,  $Pr_6O_{11}$ ; difference between total  $R_2O_3$  and  $Sm_2O_3$  curves is due to  $Gd_2O_3$ ,  $Eu_2O_3$ , etc., which do not separate cleanly from  $Sm_2O_3$  under these conditions.

Fig. 7 shows the effect of pH on the shape of the elution curves using 0.5 % citric acid and the degree of separation which can be obtained at various pH's. It will be noticed that at a pH of 4.2, while the separation is not as good as at a lower pH, it takes only a total of about 17 l. to remove the rare earths completely from the column while at a pH of 3.8 it takes as many as 47 l. It is obvious from this and other data that different complexes are involved in elution at the pH of 3.8 as against a pH of 4.2. It should also be pointed out that in general

an intermediate pH range between these two extremes may be more desirable than either of them. While the degree of separation may not be so great, the man-hours involved in making a run are much less, and the grams of pure rare earth obtained per man-month may be greater. All factors have to be considered and it may be possible to make two passes through the column at a higher pH value in the same time it takes to run one pass at the lower pH. While the separation is not as good for a single pass, at the higher pH the double pass will give better over-all separation.

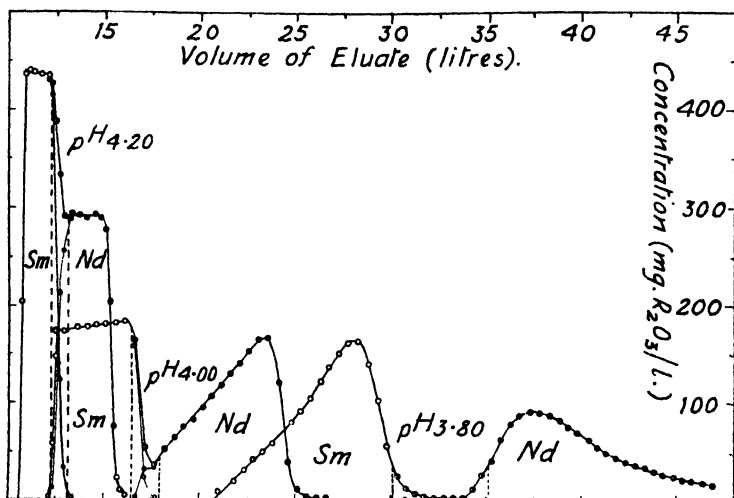


Fig. 7.—The effect of pH on the elution of 1.713-g. samples of equimolar  $\text{Sm}_2\text{O}_3$ - $\text{Nd}_2\text{O}_3$  mixtures from  $2.2 \times 120$  cm. beds of  $-30 + 40$  mesh size Amberlite IR-100 using 0.5 % citrate solutions at a linear flow-rate of 0.5 cm./min.: O,  $\text{Sm}_2\text{O}_3$ ; ●, mixed fractions; ●,  $\text{Nd}_2\text{O}_3$ .

Fig. 8 shows similar data for the praseodymium-neodymium bands under the same conditions and indicates similar conclusions.

Fig. 9 shows the effect of particle size and flow-rate on the shape of the elution curves. In general the breakthrough and the maximum of the peak occur at lower volumes of eluant with finer resins and for slower flow-rates. This is about what might be expected, since at the slower flow-rates more complete equilibrium can be obtained within the column and the diffusion rate in the solid which tends to blur the separation will be less important for finer resins.

Fig. 10 shows the effect of temperature on the shape of the elution curves. In general the breakthrough and the peak are shifted to greater volumes of eluant with increasing temperature in much the same way that a decrease in pH would shift the peaks, and part of this shift is undoubtedly due to the shifts in the equilibrium constants which occur as the temperatures change. This figure also substantiates the conclusions regarding the effect of flow-rate on the elution curves and separation factors as pointed out in Fig. 9.

Fig. 11 shows the effect of pH on the shape of the elution curves when the strength of the citric acid is dropped to 0.1 % or in other words to 1 g. citric acid monohydrate per litre of distilled water. It will be noticed that in this case sharp breakthroughs and sharp cut-offs are obtained and that there is very little trailing of the leading rare-earth elution band over the succeeding band. Also, the bands are flat-topped as might be predicted from certain types of chromatographic adsorption theory. In general, the degree of separation is better the lower the pH. On the other hand, it takes so many litres of eluant to elute the rare earths that the factors of time and cost become important. While a pH of 5.0 would probably give a better separation, we use a pH of 6.0 in most of our runs as the man-hours of labour to obtain a gram of pure rare earth are much less where a pH of 6.0 is used.

Fig. 12 shows the effect of length of column on the shape of the elution curve and the degree of separation obtained. It will be noticed that the 30-cm. column has been overloaded because we get the typical humping-up of the elution curve which is characteristic of an overloaded column. The widths of the elution bands are the same for the 60, 90 and 120 cm. columns although the amount of the eluant for the breakthrough increases with the length of the column. Therefore in this pH range there is no advantage in longer columns once the bands have attained equilibrium on the column. It also appears in this

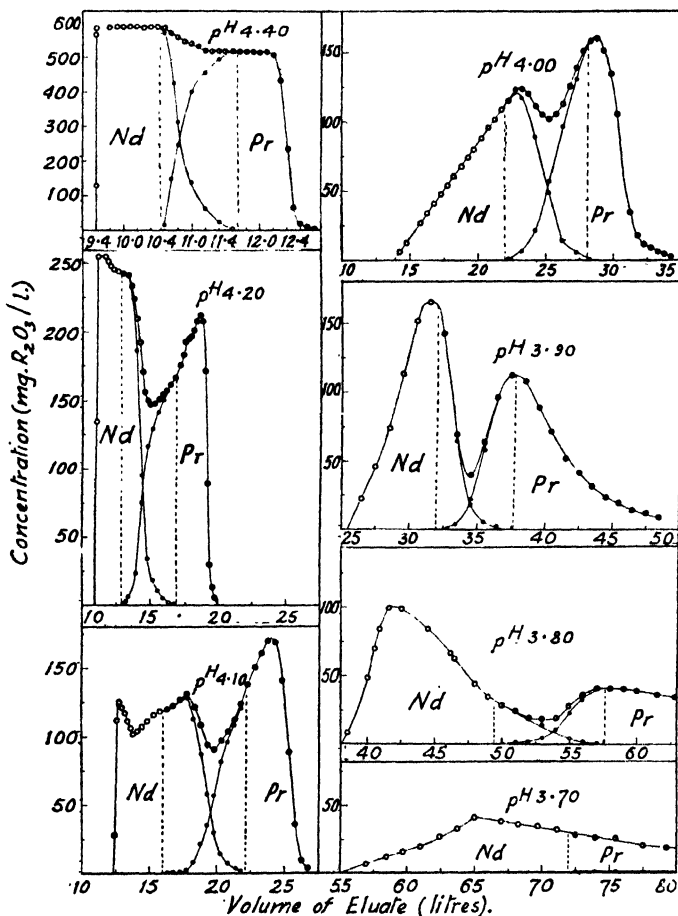


FIG. 8.—The effect of pH on the elution of 1.695-g. samples of a mixture containing equal amounts of  $\text{Nd}_2\text{O}_3$  and  $\text{Pr}_6\text{O}_{11}$  by weight from  $2.2 \times 120$  cm. beds of — 30 + 40 mesh size Amberlite IR-100 resin, using 0.5 % citrate solutions at a linear flow-rate of 0.5 cm./min: O,  $\text{Nd}_2\text{O}_3$ ; ◐, mixed fractions; ●,  $\text{Pr}_6\text{O}_{11}$ .

pH range that one rare earth replaces the other on the column and pushes it along. In other words, the bands do not travel independently of each other. These conclusions are not borne out at lower pH's and higher citrate concentrations. On the right-hand side of the graphs, we have plotted the pH values of the solutions coming off and it will be noticed that there is a break in the pH at the point where the rare-earth bands change from one species to another. We hope ultimately to take advantage of this pH break in setting up automatic controls on the columns.

## SEPARATION OF RARE-EARTH SALTS

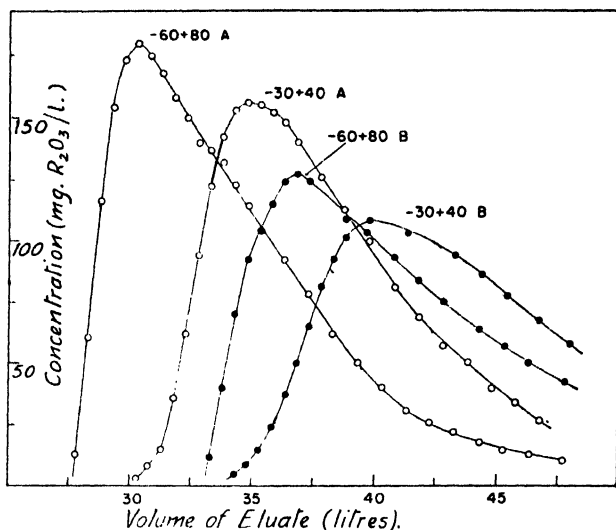


FIG. 9.—The effect of particle size of resin on the elution of 1.683-g. samples of pure  $\text{Nd}_2\text{O}_3$  from  $2.2 \times 120$  cm. beds of Amberlite IR-100 resin, using 0.5 % citrate solution at a pH of 3.80 and linear flow-rates of 0.5 cm./min. and 2.0 cm./min.: O, 0.5 cm./min.; ●, 2.0 cm./min.

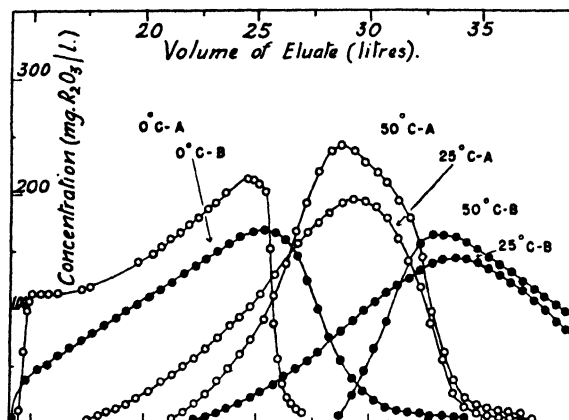


FIG. 10.—The effect of temperature on the elution of 1.714-g. samples of pure  $\text{Sm}_2\text{O}_3$  from  $2.2 \times 120$  cm. beds of -30 + 40 mesh size Amberlite IR-100 resin, using 0.5 % citrate solution at a pH value of 3.80 (measured at room temperature) and linear flow-rates of 0.5 cm./min. and 2.0 cm./min.: O, 0.5 cm./min.; ●, 2.0 cm./min.

Fig. 13 shows the separation of a mixture of three rare earths and indicates that an appreciable fraction of each of the rare earths can be obtained in a pure state with one pass through the column.

Fig. 14 shows the effect of increasing the load on the column at the same time the column is increased proportionately in length. It will be noticed that the overlap range remains about constant in all three cases. It would appear that the more heavily the columns are loaded, when the lengths of the columns are made proportionately longer, the higher the percentage of the spectrographically pure rare earths which could be obtained from a single pass through the column. Unfortunately this process cannot go on indefinitely because there seems to be an upper limit to the amount of salt which can be loaded upon a given size column.

This appears to be determined by the time it takes the bands to progress down the column. If the columns are overloaded, after a given time interval a precipitate will appear which invalidates the separation. We believe this precipitate is a citrate complex containing water of hydration which has a very slow rate of crystallization, and at the present time it is giving us considerable trouble in our separations. We have not yet tied down all the variables which determine the nature and appearance of this precipitate. However, if the loads are not too heavy it can be avoided. In our early production runs we were not much troubled with it, but after our resins became older and perhaps became seeded on the inside with this precipitate, it made its appearance more and more frequently and we are at present trying to find the cause of this precipitate and the methods by which its formation can be prevented.

At one time our columns became ineffective and we spent several non-productive months trying to find the cause. At that time we traced the difficulty to the growth of a mould in the column which prevented the separation occurring either by blocking the exchange points or by poisoning the cyclic processes due to the formation of oxalic acid or other by-products of the mould growth. This

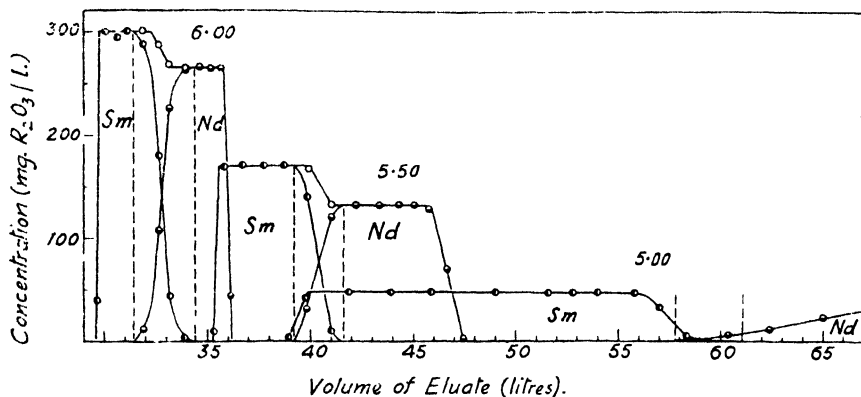


FIG. 11.—The elution of 1.713 g. equimolar mixtures of  $\text{Sm}_2\text{O}_3$  and  $\text{Nd}_2\text{O}_3$  from  $2.2 \times 120$  cm. beds of  $-30 + 40$  mesh size Amberlite IR-100 resin, using 0.1 % citrate solutions at pH values of 5.0, 5.5 and 6.0 at a linear flow-rate of 0.5 cm./min.: O, total  $\text{R}_2\text{O}_3$ ; ●,  $\text{Sm}_2\text{O}_3$ ; ○,  $\text{Nd}_2\text{O}_3$ ; broken vertical lines indicate amount of overlap between Sm and Nd bands.

difficulty was overcome by adding 0.1 % phenol to our citric acid solution. This prevented the mould from being troublesome for about two years. The moulds are again giving us trouble, however, for during this time we seem to have developed a strain of mould which can grow in a 0.1 % phenol solution. We are now faced either with the proposition of increasing the phenol concentration or of sterilizing our columns and replacing the Amberlite with fresh material.

Fig. 15 shows the effect of particle size on the separation of the rare earths at a 0.1 % citrate concentration and at appropriate pH ranges. In general, the smaller the particle size the better the separation and this might be expected because the rare earths exchanging near the surface of the particles would move down the column faster than the rare earths which exchange in the centre of the particles where the liquid has to diffuse into the centre and then out again before it can proceed down the column. Another factor which determines to a marked extent the degree of separation is how effectively channelling can be prevented in the columns. In other words, it is important that the diffusion front proceed down the column on a parallel face. If the liquid in one part of the column can flow more rapidly than in another part then this will tend to blur the separations. One might ask why we do not use fine resins exclusively and the answer



is that it is extremely difficult to get them. The resins do not grind up readily since they are semi-plastic and tend to smear into flat platelets rather than grind when put through a mill.

The pure heavy rare earths have proved much more difficult to separate than the light rare earths by the column method, just as they were more difficult to separate by the old fractional crystallization methods. In general, the equilibrium constants are closer together for adjacent rare earths in this range than they are for the lighter rare earths. Further, yttrium which is always

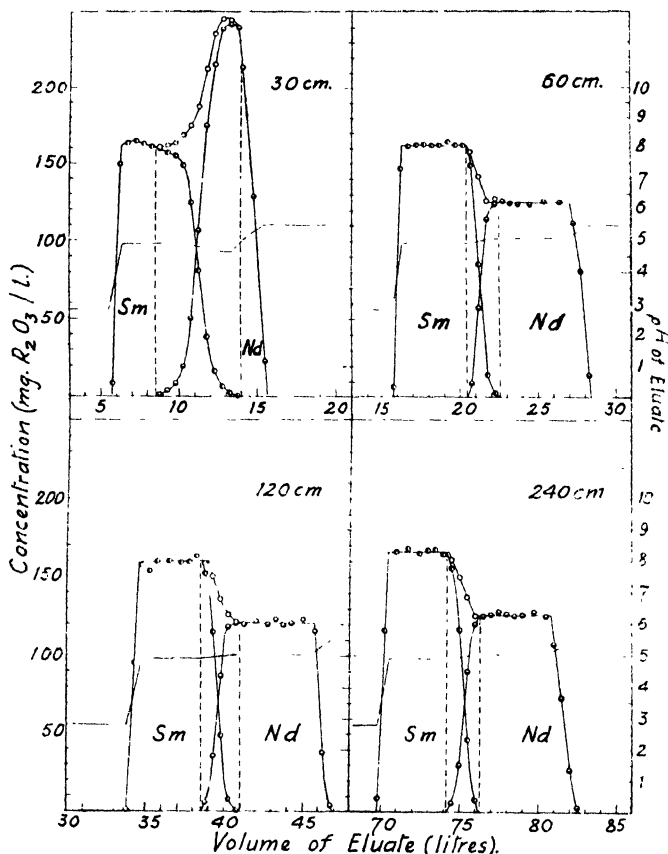


FIG. 12.—The elution of 1.713 g. equimolar  $\text{Sm}_2\text{O}_3$ - $\text{Nd}_2\text{O}_3$  mixtures from — 30 + 40 mesh size Amberlite IR-100 resin beds, 2.2 cm. diam. and 30, 60, 120 and 240 cm. long, respectively, using 0.1 % citrate solution at a pH of 5.50 and a linear flow rate of 0.5 cm./min.: O, total  $\text{R}_2\text{O}_3$ ; ●,  $\text{Sm}_2\text{O}_3$ ; ◐,  $\text{Nd}_2\text{O}_3$ ; broken vertical lines indicate amount of overlap between Sm and Nd bands; solid line across figures gives the pH of the eluate, reading on the right-hand scale.

present in large excess has an equilibrium constant almost identical with dysprosium. Another difficulty in this range is that the rare earths which can exist in the divalent state seem to have their equilibrium constants shifted in the direction of the next heavier rare earth. While praseodymium, neodymium and samarium separate very readily, it is extremely difficult to separate samarium from gadolinium. The same is true with regard to ytterbium and lutecium. Here, however, nature is kind to us in that it is possible to combine with the column technique the amalgam techniques developed by Marsh, McCoy and Yntema which take advantage of the divalent state so that separations can be obtained. Another difficulty in obtaining the heavier rare earths is that they

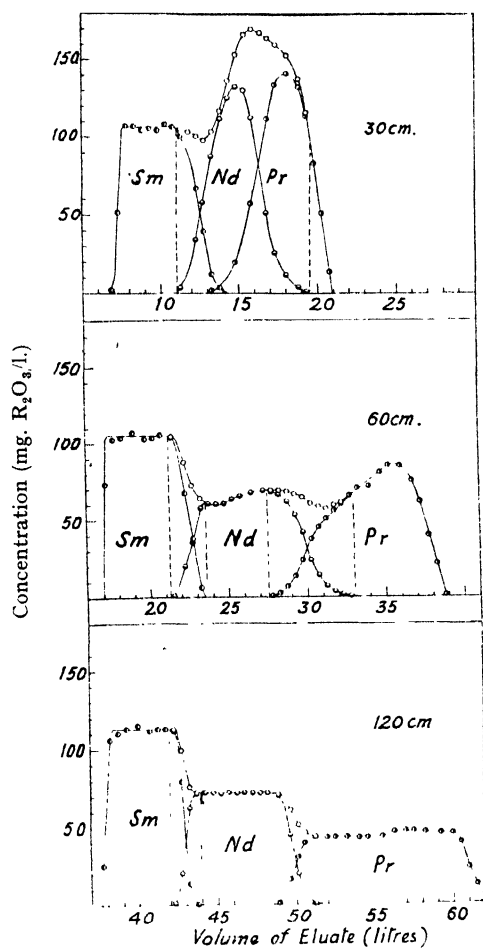


FIG. 13.—The elution of mixtures of Sm, Nd and Pr from 30 + 40 mesh size Amberlite IR-100 beds, 2.2 cm. diam. and 30, 60 and 120 cm. long, with 0.1 M citrate solution at a pH of 5.30 and a linear flow-rate of 0.5 cm./min.: O, total  $R_2O_3$ ; ●,  $Sm_2O_3$ ; ●,  $Nd_2O_3$ ; ●,  $Pr_4O_{11}$ ; broken vertical lines indicate overlap between bands.

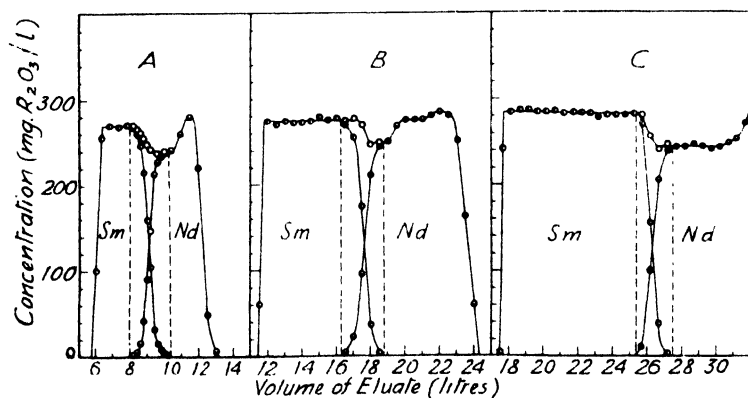


FIG. 14.—The effect of increasing column load and column length proportionately; (A) 1.65 g. of equimolar  $Sm_2O_3$ - $Nd_2O_3$  mixture on a 2.2 x 30 cm. bed of Amberlite IR-100 resin, (B) 3.30 g. on a 2.2 x 60 cm. bed and (C) 4.95 g. on a 2.2 x 90 cm. bed: O, total  $R_2O_3$ ; ●,  $Sm_2O_3$ ; ●,  $Nd_2O_3$ ; broken vertical lines indicate amount of overlap between bands.

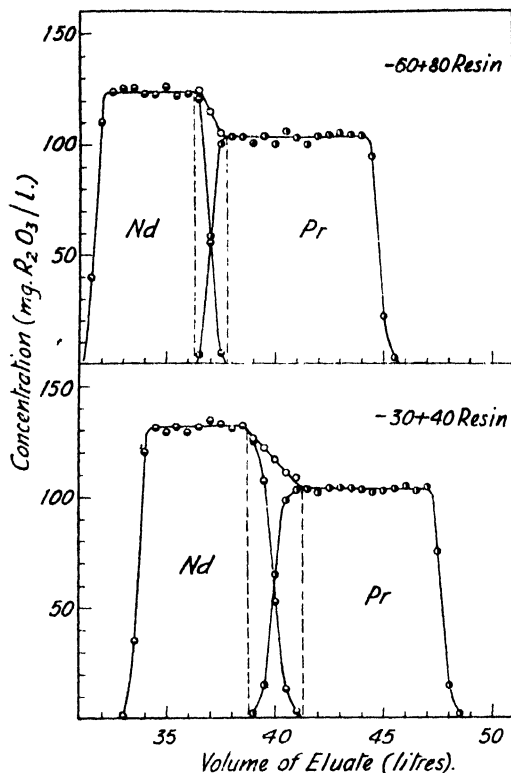


FIG. 15.—The effect of particle size of the resin on the separation of equimolar mixtures of Nd and Pr using 0.1 % citrate at a pH of 5.50 and a linear flow-rate of 0.5 cm./min.: O, total  $R_2O_3$ ; ●,  $Nd_2O_3$ ; ●,  $Pr_6O_{11}$ ; broken vertical lines indicate amount of overlap between the Nd and Pr bands.

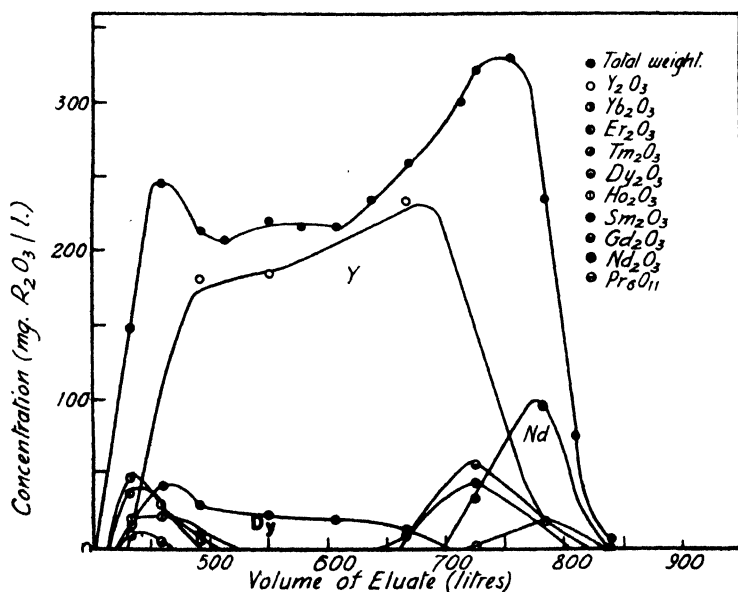


FIG. 16.—Elution of 100 g. rare-earth oxides obtained from gadolinite ore using 0.1 % citrate solution at a pH of 6.00 and a linear flow-rate of 0.5 cm./min. The column consisted of a - 30 + 40 mesh size Amberlite IR-100 bed, 4 in. diam. and 4 ft. long.

exist in nature in much smaller quantities than the lighter rare earths and considerable amounts of ore or heavy rare-earth concentrates have to be worked over in order to get very small quantities of these rarer rare earths. Our sources for obtaining the heavy rare earths have been either the ores gadolinite, blomstrandine or the crude yttrium oxalate which Lindsay has on the market.

Table III shows an approximate analysis of Lindsay's concentrates and it will be noticed that after the yttrium, cerium, neodymium and samarium have been removed only a small fraction of the concentrate remains containing the heavy rare earths.

Fig. 16 shows a typical production run on 100 g. concentrate from gadolinite ore. It will be noticed that the yttrium tends to split the rare earths roughly into two groups, the heavy and light fraction, and that the dysprosium drags across the entire yttrium band. It will also be noticed that there is a minimum in the elution curve where the yttrium occurs. This might be expected since the ordinate is plotted in mg./l. and an equal molar quantity of yttrium will contain far less grams than a similar mixture of the rare earths proper.

Fig. 17 shows a subsequent pass of the column of the heavy rare-earth fraction from the previous run. It will be noticed that there is some separation of the bands but it is not as pronounced as with the light rare earths. It is, therefore, necessary to make from four to ten passes through the columns to obtain appreciable fractions of some of these rare earths in greater than 99.5 % purity. Fewer passes would accomplish this if only two component systems were involved,

TABLE III

TYPICAL ANALYSIS OF THE LINDSAY  
"CRUDE YTTRIUM OXALATE"

Constituent	% by weight
Loss on ignition (800° C)	40
CeO <sub>2</sub>	10
Nd <sub>2</sub> O <sub>3</sub>	7
Sm <sub>2</sub> O <sub>3</sub>	5
Gd <sub>2</sub> O <sub>3</sub>	6
Y <sub>2</sub> O <sub>3</sub>	15
Dy <sub>2</sub> O <sub>3</sub>	3
Er <sub>2</sub> O <sub>3</sub>	0.2-0.5
Ho <sub>2</sub> O <sub>3</sub>	0.2-0.5
SiO <sub>2</sub>	10

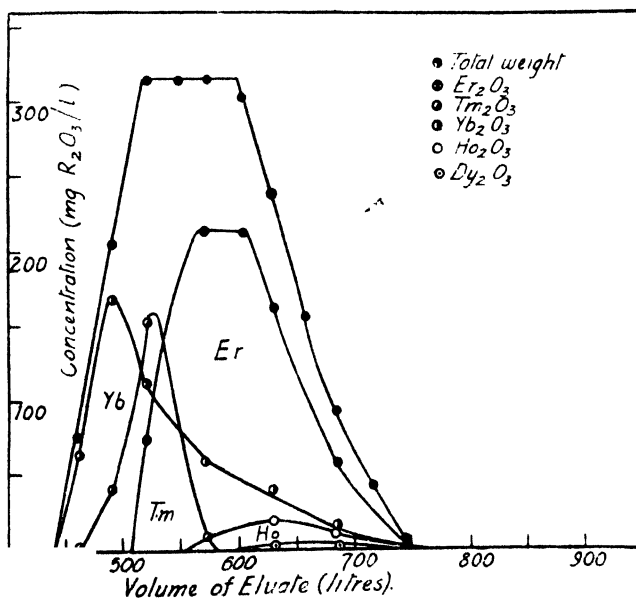


FIG. 17.—Elution of 67 g. of rare-earth oxides originally obtained from gadolinite ore using 0.1 % citrate solution at pH 6.00 at a flow-rate of 0.5 cm./min.; original composition was 20 % Yb<sub>2</sub>O<sub>3</sub>, 13 % Tm<sub>2</sub>O<sub>3</sub>, 60 % Er<sub>2</sub>O<sub>3</sub>, 5 % Ho<sub>2</sub>O<sub>3</sub> and 2 % Dy<sub>2</sub>O<sub>3</sub>.

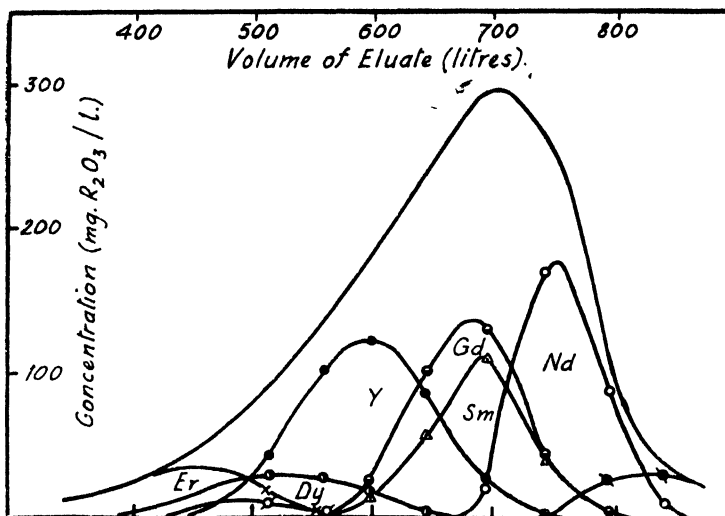


FIG. 18.—Elution curve for 100 g.  $R_2O_3$  from acid-soluble fraction of Lindsay Light and Chemical Company "yttrium oxalate" from an Amberlite IR-100 bed,  $\frac{1}{4}$  in. diam. and 8 ft. long, using 0.5 % citrate solution at a pH of 3.9 and a linear flow-rate of 0.5 cm./min.

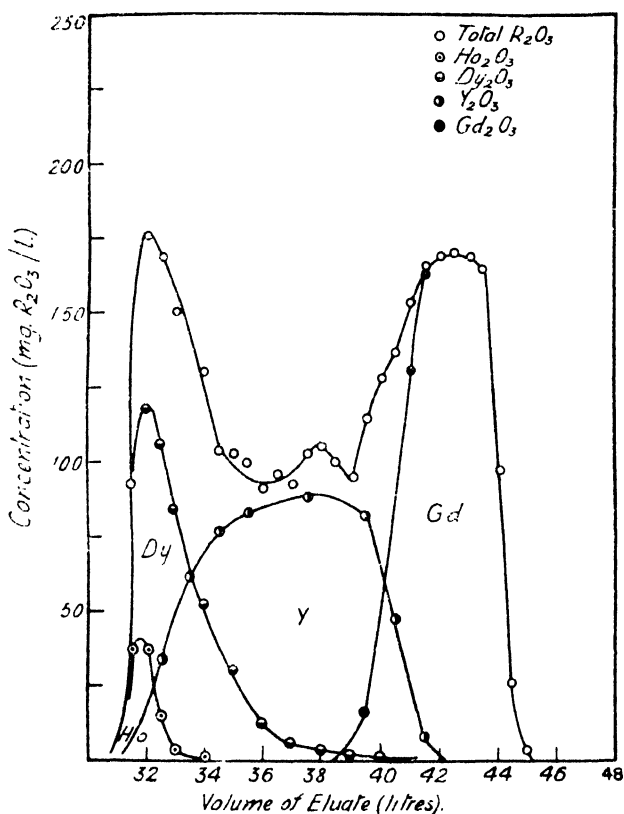


FIG. 19.—Elution of 1.5 g. mixture of Ho, Dy, Y and Gd oxides from an Amberlite IR-100 resin bed, 2.5 cm. diam. and 90 cm. long, using 0.1 % citrate solution at a pH of 5.4.

but where mixtures of 8 or 10 rare earths are to be separated more passes are necessary to separate them into their pure components.

Fig. 18 shows the positions of the elution bands for the various components when the concentrates from Lindsay's yttrium fraction were used. Fig. 19 shows the separation of holmium, dysprosium, yttrium and gadolinium when a pH of 5.4 is used for the eluant.

As an example of the success of these procedures, it might be stated that we now have about 300 g. pure ytterbium, 15 g. spectrographically pure lutecium and almost an equal amount of very rich thulium.

As mentioned earlier in the paper, we have been anxious to obtain the metals of these rare earths and we have been able to develop methods for producing them pure and in appreciable quantities. The process used is essentially the thermal reduction of the halides by means of calcium and the recasting of the metal into cylinders. This process was in the main part developed under our contracts with the Atomic Energy Commission and has not yet been declassified. At present we have a paper about ready for declassification. In the meantime it will not be possible to give further details regarding the method of reduction.

At the time of writing this paper I expect to be able to attend the symposium in England, and if this turns out to be possible I shall exhibit metal bars of pure lanthanum, cerium, praseodymium, neodymium and yttrium as well as some salts of the rarer rare earths. These metals have about the density of iron and are soft like calcium. They react readily with air and we keep them under mineral oil to protect them from corrosion. In a massive bar, while the surface corrodes readily, the rate of corrosion slows down as it penetrates the bar and it is possible, though not desirable, to keep a cylinder exposed to air for several years. It will, however, be encrusted with a thick coating of oxide which can be brushed off readily.

The work described in this paper was carried out in the Ames Laboratory of the United States Atomic Energy Commission during the past four years and the author wishes to acknowledge that it was done in collaboration with the following colleagues and graduate students: E. I. Fulmer, A. F. Voigt, B. O. Ayres, T. A. Butler, P. Figard, E. M. Gladrow, M. Gobush, P. E. Porter, J. E. Powell, N. R. Sleight, A. D. Tevebaugh, R. Q. Thompson, J. M. Wright, V. A. Fassel, D. H. Ahmann, V. Bulgrin, A. H. Daane and C. F. Miller.<sup>14</sup>

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<sup>14</sup> Spedding, Voigt, Gladrow and Sleight, *J. Amer. Chem. Soc.*, 1947, **69**, 2777. Spedding, Voigt, Gladrow, Sleight, Powell, Wright, Butler and Figard, *J. Amer. Chem. Soc.*, 1947, **69**, 2786. Spedding, Fulmer, Butler, Gladrow, Gobush, Porter, Powell and Wright, *J. Amer. Chem. Soc.*, 1947, **69**, 2812. Spedding, Fulmer, Ayres, Butler, Powell, Tevebaugh and Thompson, *J. Amer. Chem. Soc.*, 1948, **70**, 1671. Spedding, Fulmer, Butler and Powell (two papers submitted to *J. Amer. Chem. Soc.* for publication). Spedding, Daane, Ahmann, Miller and Bulgrin (unpublished work).

# APPLICATION OF ION EXCHANGE TO THE SEPARATION OF SUBSTANCES IN LOW CONCENTRATIONS

BY EDWARD R. TOMPKINS

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Ion exchange was investigated as a means of separating substances in low concentration. The programme, which began in an attempt to separate the cationic fission species without addition of isotopic carriers, was so successful that it was continued. A method and an apparatus for separating multi-Curie quantities of fission products by remote control was developed for use in producing pure radio-isotopes. This apparatus has proved useful for a number of other separations in the radio-isotope production programmes of the United States Atomic Energy Commission.

The separation of rare earths was studied by both equilibrium and column methods. Conditions which were satisfactory to separate rare earths, scandium and alkaline earths in very high purities were established by studying the effect of a number of variables on the distribution coefficient and the shape of the elution curves.

The "plate" theory was applied to ascertain the degree of separation of two or more solutes eluted from a column. By determining the column constants and the equilibrium distribution coefficients of two solutes, it was possible to calculate elution curves which closely approximated the curves determined experimentally. The theoretical implications and the general applicability of ion exchange for difficult separations are discussed.

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**Introduction.**—In attempting to devise methods for separating the fission elements without the addition of isotopic carriers, the ion-exchange technique was investigated. Several groups connected with the United States Atomic Energy programme were active in this programme in various capacities. This summary will be limited to the contributions of the group consisting of W. E. Cohn, D. H. Harris, J. X. Khym, G. W. Parker, S. W. Mayer and E. R. Tompkins.<sup>1-8</sup>

In the early experiments the mixture of fission products was adsorbed in a narrow band at the top of a column of Amberlite IR-1 exchanger, and subsequently, the fission products were eluted from the column by replacement with an alkali salt or a strong acid of 0.1 to 1 M concentration. Although the early analytical methods were inadequate, it was obvious from the differential radioactive measurements on aliquots of the eluate that the separations were incomplete.

At about this time, E. R. Russell<sup>9</sup> demonstrated that the fission zirconium and columbium could be desorbed selectively from an Amberlite IR-1 bed upon which a fission product mixture had been adsorbed by passing a dilute solution of oxalic acid through the column. These observations suggested the possibility of using other reagents which would form soluble

<sup>1</sup> Tompkins, Khym and Cohn, *J. Amer. Chem. Soc.*, 1947, **69**, 2769.

<sup>2</sup> Harris and Tompkins, *J. Amer. Chem. Soc.*, 1947, **69**, 2792.

<sup>3</sup> Tompkins and Mayer, *J. Amer. Chem. Soc.*, 1947, **69**, 2859.

<sup>4</sup> Mayer and Tompkins, *J. Amer. Chem. Soc.*, 1947, **69**, 2866.

<sup>5</sup> Tompkins, *J. Amer. Chem. Soc.*, 1948, **70**, 3520.

<sup>6</sup> Cohn, Parker and Tompkins, *Nucleonics*, 1948, **3**, 22.

<sup>7</sup> Tompkins, *J. Chem. Educ.*, 1949, **26**, 32, 92.

<sup>8</sup> Tompkins, Harris and Khym, *J. Amer. Chem. Soc.*, 1949, **71**, 2504.

<sup>9</sup> Russell (unpublished work).

co-ordination compounds with the various fission elements. It appeared that it might be possible to select reagents which would selectively elute the fission products in groups (i.e., columbium and zirconium, alkaline earths, rare earths, alkali metals, etc.). Because of the ability of citrate and tartrate to form soluble alkaline earth complexes, they were chosen for the initial investigations. Citrate was employed more extensively than tartrate since the greater solubility of its salts permitted studies over a wider range of concentrations.

Preliminary experiments showed that citric acid in a concentration of 0.23 M would not remove a measurable fraction of any of the fission elements from Amberlite IR-1 after columbium and zirconium had been eluted with oxalic acid. Tri-ammonium citrate was tried next and it was found to remove not only the alkaline earths but also the other fission elements as nearly quantitatively as could be determined by preliminary radioactive measurements. Investigations employing citrate solutions of pH's between that of citric acid (about 1.8) and that of tri-ammonium citrate (about 7.5) showed that a fair degree of separation of all the cationic fission products, including the rare-earth elements, could be effected with this one reagent.<sup>1</sup>

In attempting to improve the separations, particularly of the rare earths, further studies were carried on employing both the column procedure and equilibrium methods. The results of most of these investigations have been reported elsewhere.<sup>2-8</sup> This account will summarize them and give the more important conclusions which have been drawn from these studies, particularly as they apply to the separations of rare-earth and alkaline-earth elements.

**Factors Affecting Separation.**—The degree of separation of similar solute ions, such as adjacent rare earths, when they are eluted from a column is determined by (i) the separation for each exchange (i.e., the equilibrium separation factor) and (ii) the average number of exchanges that the ions make as they travel down the column.<sup>4</sup> Variables affecting the separation factors may be studied most readily by equilibrium experiments while those that determine the number of exchanges must be studied by column experiments.

**Factors Affecting the Equilibrium Separation of Solutes.**—A number of factors which affect the separation of solutes were studied by equilibrium experiments.<sup>3</sup> In most of these experiments, a known mass of exchange resin in the ammonium form was shaken with a measured volume of solution of known composition. Radio-isotopes of the solutes being investigated (usually fission products) were employed for facilitating the analyses. Radioactive measurements to determine the changes in the solute concentrations in the solution phase permitted the calculation of the distribution coefficients. There was no appreciable error in the use of this technique since the total solute concentration was usually very low.

A comparison of several exchangers as to their effectiveness in separating adjacent rare-earth elements showed considerable variation.<sup>8</sup> Synthetic resins with methylene sulphonic acid groups were less effective than those with nuclear sulphonic acid groups. Of the latter group, the sulphonated hydrocarbons were superior to sulphonated phenol-formaldehydes.

There is ample evidence from both the equilibrium and column experiments to show that separation is most efficient when the concentrations of the solutes are low.<sup>1,2,3,8</sup> For ionic concentrations below a certain critical value, which varies with the type of exchange resin employed (e.g.,  $\sim 10^{-7}$  M rare earths for Amberlite IR-1 and  $\sim 10^{-4}$  M rare earths for Dowex 50), changes in the solute concentration do not affect the distribution coefficients of the solutes between the solid and liquid phases appreciably, and thus do not alter their separation.<sup>3</sup> At higher ionic concentrations, the distribution



coefficient of each solute is reduced, the net effect being a decrease in their separation. In this respect, the ion-exchange and adsorption methods of separating substances are unique since, unlike most other methods, they are more effective at very low ionic concentrations than for macroscopic quantities of substances.

Complexing agents serve a double purpose in increasing the separation of similar solutes, particularly when they are present in macroscopic quantities. Such substances not only contribute to the separation due to the differences in the dissociation constants of their co-ordination compounds with the solutes, but they also reduce the fractions of the solutes in the ionic form and thus their ionic concentrations. This permits larger quantities of solutes to be separated without exceeding the ionic concentrations above which the separation is affected adversely.

Because of the initial success in separating similar elements by use of citrates, it has been studied more extensively than other complexing agents. Preliminary studies indicated that several other compounds such as tartrates, lactates, sulphosalicylates and acetylacetonates were equally effective or superior to citrates for separating rare earths.<sup>3</sup>

The ideal situation would be to have a specific complexing agent for each substance which would form a very strong complex with it and not form a complex with any other substance. This would permit any mixture of ions to be separated by adsorbing them on an exchanger and subsequently equilibrating the exchanger successively with solutions each containing the complexing agents specific for a single substance.<sup>7</sup> Although this is applicable for some mixtures of dissimilar substances, it cannot be used for separating very similar ions such as the rare earths. A complexing agent for this application should form a water-soluble co-ordination compound with a dissociation constant in the range <sup>3 4</sup> of  $10^{-4}$  to  $10^{-6}$ .

Complexing agents which must be ionized in order to form co-ordination compounds with solutes are affected by changes in pH which determine the fraction in the ionized form. If they have more than one dissociable hydrogen (e.g., citric and tartaric acids), several complexes with the solutes are possible. For separating rare earths the complex with the di-hydrogen citrate ion proved most effective, the separation factor decreasing as the pH was increased.<sup>1 2 3</sup> The alkaline earths were separated most readily at pH 5 to 8, indicating that the completely dissociated citrate ion was functioning.<sup>1 5 6</sup>

The relative strengths of the bonds between an exchanger and similar solutes depend upon the hydrated radii of the solute ions, while the relative strengths of the complexes usually depend upon their non-hydrated radii. The hydrated radius of the rare-earth series increases and the non-hydrated radius decreases with increasing atomic number. Thus the separation effects of the exchanger and the complexing agent supplement each other, the former acting through variations in the relative bond strengths and the latter by varying the relative ionic concentrations of the rare earths due to different degrees of complex formation. Studies in which a sulphonated hydrocarbon resin (Dowex 50) was equilibrated with solutions of rare-earth salts with and without citrate indicated that the separation due to the exchanger and to the complexing agent were of about equal importance.<sup>3</sup> As would be expected, studies with alkaline earths showed the reverse relationship between the atomic numbers and the distribution coefficients of solutes between the citrate solution and the exchanger, radium having the highest distribution coefficient.<sup>1 5 6</sup>

**Factors Affecting Column Efficiency.**—An exchange resin consists of a highly cross-linked organic matrix with attached sulphonic acid groups, distributed uniformly. However, since the resins are in the form of discrete

particles, the column bed is not homogeneous. To exchange with another ion attached at the surface of a particle an ion in a solution flowing through the column needs only to diffuse through the immobile liquid layer surrounding it. Exchange of an ion within a resin particle is dependent upon ionic diffusion through the resin.

If the flow-rate of solution through a column is sufficiently slow and the particles of exchanger are very small, the differences between the behaviour of the ions at various distances from the surface of the particles will be minimized.<sup>4 7 8</sup> Increasing the rate of diffusion by raising the temperature likewise will result in a closer approach to equilibrium.<sup>10</sup>

If a very small quantity of solute ions are first adsorbed in a narrow band at the top of a column and then a solution containing other ions passed through the column, a portion of the solute ions will be replaced from their original resin attachments and be carried down the column by the solution so that they come into contact with fresh exchanger. As each solute ion in solution passes an exchange position, there is a certain probability that it will exchange.<sup>4 7</sup>

When a column is operated in such a manner that equilibrium conditions are approached, the solute ions will travel a certain average distance through the column before exchanging. For each exchange, the separation between solutes will approach the equilibrium separation factor.<sup>4 7</sup> Therefore, for purposes of calculating separations of solutes, a column may be considered as being made up of a number of "batches" of exchanger, each successively equilibrating with portions of solution of volumes sufficient to occupy the void spaces around the particles of exchanger. To adopt more commonly used terminology, these elements of the column may be referred to as "plates."

The application of the "plate" theory to ion-exchange column separations<sup>4</sup> is very similar to its application to partition chromatography.<sup>11</sup> For this reason it will not be discussed here. Its applicability for analyzing individual elution curves and for calculating the degree of separation of solutes with overlapping elution curves has been demonstrated amply.<sup>4 7</sup> By determining the equilibrium distribution coefficients of any two solutes and their separate elution curves from a short column, it is possible to predict accurately how much eluent must be passed through a column of any other size before solute first appears in a measurable concentration in the eluate. Furthermore, the calculations are simple and the method is easy to apply, especially if the analyses are made by radioactive techniques.<sup>7</sup>

Any factor which changes the average distance a solute ion travels down a column between exchanges will affect the column efficiency. Thus the flow-rate is of prime importance since, as it is increased, the ions are carried at a faster rate down the column and so have less time to diffuse to an exchange position. The size of the resin particles determines the size of the liquid interstices between them and thus the average distance an ion must diffuse to reach the particle. Temperature which increases the diffusion rate increases the number of exchanges in a given column length.<sup>10</sup> Likewise, the exchange capacity of the resin (i.e., the number of exchange positions per unit weight) affects the efficiency.<sup>4</sup> The sulphonated hydrocarbons with exchange capacities of about 6 m.-equiv./g. are far superior to the sulphonated phenol-formaldehydes with capacities of one-third to one-half of this amount.<sup>2 3 5 8 10</sup>

In addition to these factors, the distribution coefficient affects the column efficiency since it determines the fraction of time the ions are in solution

<sup>10</sup> Ketelle and Boyd, *J. Amer. Chem. Soc.*, 1947, **69**, 2800.

<sup>11</sup> Martin and Synge, *Biochem. J.*, 1941, **35**, 1385.

and thus the distance they will be carried down the column between exchanges. The use of 0.23 M citrate solutions at pH above 3.0 (or higher citrate concentrations at somewhat lower pH) tends to give poor separations of rare earths.<sup>1 2 3 8</sup> Likewise, the use of high concentrations of strong acids and solutions of their salts give poor separations of similar solutes.

All of the factors discussed above, which affect column efficiency, are interdependent.<sup>7</sup> For example, decreasing the flow-rate will compensate for increasing the resin particle size, the use of an elutrient which will give a low distribution coefficient, etc.<sup>4 8</sup> For this reason, it is difficult to study the effect of column variables on efficiency. A large quantity of data was accumulated without adequate control of the different variables before this fact became apparent.<sup>1 2 4 6 8</sup> Although they were helpful in establishing optimum operating conditions for some separations, they are of doubtful value for theoretical interpretations.

**Factors which Affect the Shape of Elution Curves.**—The theoretical elution curves for solutes in low concentrations approach the normal curve of error.<sup>4 11</sup> Deviations in form from the theoretical curves usually indicate decreases in separation.<sup>1 2 4 6 8</sup> The most common form of elution curve is that which is asymmetrical with a steeper slope on the leading than the trailing edge.

The following are a few of many causes for asymmetry in elution curves :

(i) Too rapid a flow-rate and/or too large particles of exchanger will cause a graded behaviour of ions depending on their positions in the resin particles. This results in a long trailing edge of the elution curves and thus in an increased overlap of curves for similar solutes.

(ii) A quantity of solutes so high or an elutrient which will give a low enough distribution coefficient that the concentration of solute exceeds that value above which the distribution coefficient is affected will produce asymmetry of elution curves. This condition usually prevails in only that portion of the column having the highest concentration of solutes. This causes all similar solutes to move more rapidly in this zone, thus giving asymmetrical curves with steep leading edges and decreased distances between them.<sup>8</sup>

(iii) When a complexing agent such as ammonium di-hydrogen citrate is used to elute a rare earth from an exchanger which is principally in the hydrogen form, a pH boundary will be formed in the column.<sup>6 8</sup> This is caused by the conversion of the hydrogen-form resin to the more stable ammonium form. But the citrate is thereby converted to citric acid which has a very low concentration of complex-forming citrate ions. Thus the solute ions are re-adsorbed from the citrate solution at this boundary. If the pH of the elutrient is sufficiently high to cause the solutes to move faster than the pH boundary, they will concentrate at that boundary and be eluted in high concentration, when this boundary reaches the bottom of the column, giving curves whose degrees of asymmetry depend upon the relative rates of the boundary and the solutes. If, on the other hand, the pH boundary moves down the column faster than any of the solutes, their elution curves will be of the same form as though they had been eluted from a column of exchanger originally in the ammonium form.<sup>8</sup>

In addition to decreasing the separation, conditions causing asymmetrical elution curves do not lend themselves readily to calculations of the probable degree of separation. For this reason, it usually is preferable to choose operating conditions which will give symmetrical elution curves, particularly in exploratory work.

**Conclusions.**—Applications of the method developed for separating fission elements by members of the group named above have resulted in the following :

(i) The separation, concentration, purification and metathesis of multi-Curie quantities of each cationic-fission product, including the rare-earth elements, with specific activities as high as several Curies per milligram of total solids and in radiochemical purities greater than 99.9 %.

(ii) Separation and purification of gram to kilogram quantities of each rare earth in purities greater than 99.9 % and in some cases with estimated purities greater than 99.99 % (upper limits of impurities determined by activation analysis). Also, it was demonstrated that adjacent rare earths differing in concentration by a factor of  $10^9$  could be separated with approximately the same efficiency as when they were both present in comparable concentrations.

(iii) Purification of several hundred grams of scandium to such a degree that the levels of impurities were below detection even by means of activation analysis.

(iv) Separation of macroscopic quantities of strontium, barium and radium in high purities.

(v) Demonstration of the applicability of this method for separating similar substances of many kinds both organic and inorganic.

The experimental work, the results of which are summarized here, was performed at Oak Ridge National Laboratory, Oak Ridge, Tennessee.

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## GENERAL DISCUSSION

**Dr. B. A. J. Lister** (*A.E.R.E., Harwell*) said: The mechanism of adsorption of cations on alumina described by Dr. Sacconi is interesting to me in view of some tests I carried out to study the effect on the adsorption series of replacing water as solvent by a non-ionizing solvent, dioxan. I examined the adsorption of nine cations:  $\text{Fe}^{3+}$ ,  $\text{Ni}^{2+}$ ,  $\text{Ag}^+$ ,  $\text{Co}^{2+}$ ,  $\text{Cr}^{3+}$ ,  $\text{Zn}^{2+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Cd}^{2+}$  and  $\text{Ce}^{3+}$  and found the order of preference considerably different from that found in aqueous solution or with any of the tried complexing agents.

DIOXAN	WATER	AMMONIA	TARTRATE
$\text{Fe}^{3+}$ , $\text{Ni}^{2+}$	$\text{Fe}^{3+}$ , $\text{Cr}^{3+}$	$\text{Co}^{2+}$	$\text{Cd}^{2+}$
$\text{Ag}^+$ , $\text{Co}^{2+}$ , $\text{Cr}^{3+}$	$\text{Cu}^{2+}$	$\text{Zn}^{2+}$	$\text{Cu}^{2+}$ , $\text{Zn}^{2+}$
$\text{Zn}^{2+}$	$\text{Ag}^+$	$\text{Cd}^{3+}$ , $\text{Cu}^{2+}$	$\text{Fe}^{3+}$ , $\text{Cr}^{3+}$
$\text{Cu}^{2+}$	$\text{Zn}^{2+}$	$\text{Ni}^{2+}$	$\text{Co}^{2+}$
$\text{Cd}^{2+}$ , $\text{Ce}^{3+}$	$\text{Cd}^{2+}$	$\text{Ag}^+$	$\text{Ni}^{2+}$
	$\text{Ni}^{2+}$		
	$\text{Co}^{2+}$		

For example, the order of adsorption of  $\text{Cu}^{2+}$  and  $\text{Co}^{2+}$  ions was reversed and only reverted to the normal order for aqueous solution when there was about 5 % water present. Similarly in dioxan solution, silver is more strongly held than copper whereas the reverse is the case in aqueous solution. In the dilute salt solutions used there was generally a trace of water present from the hydrated salt but this was never  $> 0.5$  %. From the colour of the solutions of the nitrates of copper, cobalt, nickel, etc., in dioxan and from the colour of the adsorbed bands it seems probable that the metals are present not in ionized form but as neutral molecules both in solution and in the adsorbed state.

In this case the hypothesis of the water providing the adsorption link is perhaps modified. The cupric ion, for example, has 4 water molecules in the

inner hydration shell, but possibly in a non-ionizing solvent where the copper nitrate may occur as neutral molecules there are fewer water molecules per copper atom. Incidentally I may add that, contrary to Dr. Flood's experience, we have never found silver to be more strongly adsorbed than copper from aqueous solution.

**Dr. L. Sacconi** (*Florence*) (*communicated*): The hypothesis of a hydrolytic adsorption of electrolytes on alumina holds, of course, only for aqueous solutions of aquo-ions. The adsorption of inorganic salts from solutions in non-ionizing solvents is probably molecular. Anhydrous  $\text{FeCl}_3$  dissolved in benzene or toluene, e.g., is firmly adsorbed on an alumina column forming a yellowish-brown band. Development with  $\text{KCNS}$  or  $\text{K}_4[\text{Fe}(\text{CN})_6]$  solution has no effect. The red or blue colour produced by  $\text{Fe}^{+++}$  ions takes place only after washing with  $\text{HCl}$  solution, which evidently splits off  $\text{Fe}^{+++}$  ions from the adsorbed undissociated  $\text{FeCl}_3$ .

The different order of preference of many cations from the different solutions, according to the different solvents and complexing agents employed, confirms that atomic groups co-ordinated around cations play a part in chromatographic adsorption. This is also confirmed by the results reported by Dr. Lister about the influence of water mixed with dioxan. It is evident that 5 % water is enough to form aquo-ions from the dissolved  $\text{Cu}$  and  $\text{Co}$  salts.

**Mr. R. A. Wells** (*Teddington*) said: In answer to Dr. Angell's question regarding the lower limit of concentration of nickel which may be estimated by the cellulose column method, we have made no accurate measurements of the limits of the process, but from our experience there appears to be no difficulty in separating very large amounts of cobalt and copper from small amounts of nickel. Dr. Davies informs me that he has estimated, semi-quantitatively on a paper strip, 0.03 % of nickel in cobalt metal.

Dr. Pollard mentions the difficulty of detecting the alkali metals on paper strips. The following method may be of interest: we have found when using a neutral chloride solution for the separation of lithium, sodium and potassium that the metals move as their salts; thus, by testing the strip for chloride, by spraying with silver nitrate and warming it has been possible to locate the position of the cations.

**Dr. F. H. Pollard** (*Bristol*) (*communicated*): It should be pointed out that the kojic acid oxine spraying reagent which we describe in our paper is satisfactory for the identification of the position of the alkali-metal ions.

With regard to the test described by Mr. Wells, we should like to observe that in our experience and that of Westall<sup>1</sup> the anions may under some conditions travel independently of the cations present on the filter paper. Thus it would seem that any test for a cation which depends on the presence of the anion at the same spot must be used with caution.

**Dr. E. Glueckauf** (*A.E.R.E., Harwell*) said: Mr. G. Robinson mentions that the 8-hydroxyquinoline columns cannot be applied to steel analysis on account of the large amount of iron present. This difficulty can be obviated by removal of the iron by partition chromatography on Highflow Super-cel saturated with 6.5 M  $\text{HCl}$  (aqueous). The steel, dissolved in  $\text{HCl}$ , is concentrated as much as possible and the  $\text{HCl}$  content is brought up to 6.5 M. This solution is put on top of the column, and then developed with ether previously shaken with 6.5 M  $\text{HCl}$  (aqueous). (This figure is fairly critical.) Instead of ether, isopropyl ether may be used with advantage. Under these circumstances only iron will be found in the ether eluate, while all the other metals remain in the column. They can subsequently be eluted with aqueous solutions and subjected to the 8-hydroxyquinoline treatment.

**Mr. G. Robinson** (*Sheffield*) said: Dr. Glueckauf's transfer of the familiar Rothé ether separation to the chromatographic column is a masterly adaptation of an accepted technique to modern methods. At first sight it appears to be an ideal method of eliminating iron from steel solutions and I shall be happy to try out the method prior to adsorption on an 8-hydroxyquinoline column.

<sup>1</sup> Westall, *Biochem. J.*, 1948, **42**, 249.

**Prof. L. Zechmeister** (*California*) said : It is proposed that the brush method as applied in collaboration with Dr. Frehden<sup>2</sup> to the detection of certain metals on the alumina column could be further developed. By making use of a special brush (dipped in a specific colour reagent) for each metal and by drawing parallel streaks along the column, the respective metals can be easily detected, even if no clean geometrical separation had taken place.

**Mr. G. Robinson** (*Sheffield*) said : I have used a technique which involves the application of dimethylglyoxime as a specific reagent for nickel in a manner analogous in some respect to Prof. Zechmeister's brush method. If one pre-coats alumina with dimethylglyoxime from alcoholic solution and then prepares a column from the dried material it is possible to pass an alkaline solution of a steel (with the iron complexed as tartrate) through the column and obtain a pink band due to nickel at the top. The length of band is proportional to the nickel content and by use of suitable standards one can arrive at an approximation of the nickel percentage.

Similar results for copper in non-ferrous alloys can be obtained on a column of alumina pre-treated with sodium di-ethyldithiocarbamate.

**Prof. A. Tiselius** (*Uppsala*) said : It ought to be quite interesting to try the application of displacement development in the separation of ions on the ion-exchange resins, on alumina (Schwab) or on alumina-impregnated filter paper (Flood). The experiments of Partridge<sup>3</sup> demonstrate the application of this procedure to some organic bases and amino acids, and it is obvious from the results of Flood and Schwab that a strong mutual displacement often takes place, and that addition of a displacement agent in a suitable concentration is likely to make the separation more complete. Thus it may be possible to utilize the capacity of the columns (i.e., the filter paper) to its full extent.

Also, I would like to ask what is considered to be the most favourable dimensions of columns, both for ion-exchange and other types of chromatography.

**Dr. E. Glueckauf** (*A.E.R.E., Harwell*) said : The important question : what is the optimum length/diameter ratio for a column is to some extent answered by eqn. (3a, b, c) and (4a)\* which contain the variable  $A$ , giving the amount of

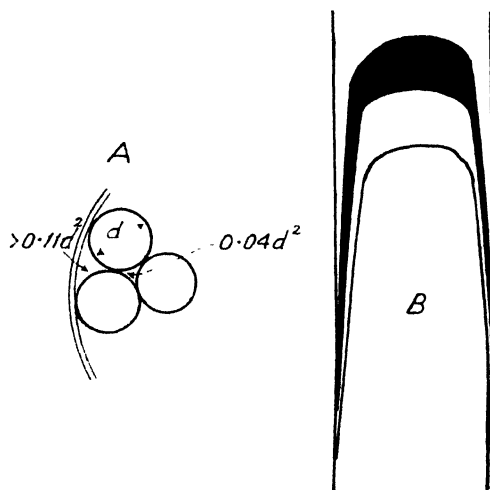


FIG. 1. Wall-channelling due to difference in pore areas.

adsorbent per cm. length of column. We see that  $A$  enters only in those cases where finite grain size and longitudinal diffusion are the disturbing factors, and not in the case of non-equilibrium. In the latter case the length/diameter ratio is

<sup>2</sup> *Bull. Soc. chim. Biol.*, 1940, **22**, 458.

<sup>3</sup> This Discussion.

\* See p. 202

therefore immaterial, while in the former case decreased diameter offers advantages provided that the total amount of sorbent in the column is constant. But some reservations must be made due to features which are not considered in these equations. If the column is too short and fat, disturbances due to channelling and tilting of the boundaries become predominant, effects which cannot be assessed numerically.

If the column chosen is too thin and long then the area of the larger pores at the wall (see Fig. A) becomes an appreciable fraction of the total cross-sectional area of the column and severe wall-channelling arises leading to a boundary form in the column of the type (Fig. B) which causes mixing of the different separated solutes in the eluate.

This effect disappears again when extremely thin micro-columns, with diameters which are a small multiple of the grain diameter only, are used. In this case, lateral diffusion and convection are sufficiently effective in turning the sharp but tilted boundaries into diffuse horizontal ones, which can be sharpened up again by the chromatographic process. We thus see that there must be a range of column diameter somewhere between, say, 5 and 50 grain diameters which is less favourable for chromatographic separations, but it does not seem possible to define this region precisely.

For substances difficult to separate ( $(K - 1) < 0.1$ ) extra allowance must be made for the fact that the boundaries spread over a considerable length depending on the values of  $(K - 1)$  and on the height of the theoretical unit  $\Delta/A$ . In order to obtain a pure product ( $> 99\%$ ) the length of the column must exceed the value  $\left(\frac{K}{K-1}\right)^2 \frac{\Delta}{A}$  by a considerable factor ( $> 30$ ) depending on the ratio  $C_1^\circ/C_2^\circ$ .

Thus we find that whilst there are quite a number of considerations which have to be weighed against each other, it is usually possible to make reasonable estimates of the required column conditions beforehand.

**Dr. W. M. Smit** (*Amsterdam*) said: In connection with this it is noteworthy that we found experimentally that in development the separation efficiency of a column is not affected if the weight ratio sample/adsorbent is kept constant and the column is properly packed.

**Dr. R. Lessing** (*London*) said: There is a close analogy between the problem of dimensioning adsorption columns and the chemical engineer's problem in designing packed towers and selecting the best combination of diameter, height, type and size of packing elements. The complexity of the disturbing factors militates against the achievement of the perfect design, and one cannot hold out much hope that the desire of Prof. Tiselius to find the ideal dimensions of an adsorption column will find a satisfactory answer in the foreseeable future.

**Dr. E. Glueckauf** (*A.E.R.E., Harwell*) said: Dr. Synge asked why in Fig. 9 in Spedding's paper the increase of grain size results, in addition to the usual broadening of the bands, also in an increase of the breakthrough volume. The reason for this is that here we have solute in bulk and not in tracer quantities. Consequently we have a curved isotherm which is also shown by the asymmetrical form of the bands. The self-sharpening front of such a band moves with a velocity which is the greater, the higher the frontal concentration. Larger grain size results in less complete equilibrium which leads to a greater spreading of the band. This involves decrease of the frontal concentration, and hence, for curved isotherms, a reduced travel rate of the front. This effect cannot occur at tracer concentrations because there we have a linear isotherm.

**Mr. C. D. Cook** (*Widnes*) said: Using a *n*-hexane-nitromethane-silica system and solutes of linear isotherms, I have found that, in addition to the factors influencing the asymmetry of the elution curves enumerated by Dr. Tompkins, one of the major factors was in the silica itself.

Having examined a large number of commercial silicas, and silicas prepared in the laboratory under differing conditions, it was found that the silicas could be divided into two groups:

- (a) silicas in which the elution curves were approximately symmetrical ;
- (b) silicas in which the elution curves were asymmetrical. The elution curves usually exhibited a right-hand skew.

It has been found possible to predict whether or not the elution curves will exhibit asymmetry from the appearance of the silica under the electron microscope. I hope to have more to say on the characterization of silicas and the dependence of reproducibility of the elution curves on the type of silica, using the electron microscope, in the near future.

Impure nitromethane, used as the immobile phase, containing higher nitro-paraffins, resulted in asymmetrical elution curves. Loosely packed columns, in which the gel volume decreases during elution, usually results in asymmetrical elution curves.

It is essential that rigid gel packing techniques should be followed when preparing a column for elution purposes. We have found that it is possible to reproduce gel volumes to within  $\pm 5$  ml., and for some silicas even within  $\pm 3$  ml., on 200–300 ml. columns when silicas from the same batch are used.

**Prof. C. W. Davies** (*Aberystwyth*) said : Mr. Nancollas at Aberystwyth has studied carbonate-hydroxide equilibria on the strong base resin Amberlite IRA-400. As would be expected, the divalent ion is preferentially held by the resin. This can be turned to good account in the preparation of  $\text{CO}_2$ -free alkali, as the whole of the carbonate can be removed when a caustic soda solution, prepared without special precautions, is run through a suitable column of the resin.

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## II(B). ORGANIC AND BIOCHEMICAL

### THE CHROMATOGRAPHY OF GASES AND VAPOURS

BY C. S. G. PHILLIPS

*Received 14th July, 1949*

A simple self-recording apparatus is described for the analysis of gas and vapour mixtures by displacement from an adsorbent. The influence of factors, such as particle size, flow-rate, column design, type of adsorbent, temperature and nature of the mixture, upon the accuracy of the analysis is discussed. Illustrations are given of the application of the method.

The separation of gas and vapour mixtures by fractional desorption from an adsorbent has been investigated by a number of workers. A list of references is given by Claesson.<sup>1</sup> Two general methods have been employed. In the first the gases are removed from the adsorbent by driving a heater along the column. This method has recently been applied by Turner,<sup>2</sup> and an instrument employing the principle is now available in America for the analysis of gas mixtures containing light hydrocarbons.<sup>3</sup> In the second method, an inert carrier gas is passed through the column of adsorbent, and plays a part similar to that played by the solvent in liquid chromatography. The various components of the mixture are desorbed one by one, to produce an "elution" curve as in Fig. 1A. This method has been considerably improved by Claesson,<sup>1</sup> who employed a carrier gas containing a constant concentra-

<sup>1</sup> Claesson, *Arkiv. Kemi, Min. Geol. A*, 1946, **23**, No. 1.

<sup>2</sup> Turner, *Oil Gas J.*, 29th April, 1943; *Petroleum Refiner*, May, 1943.

<sup>3</sup> *Bull. No. 205, Burrell Technical Supply Co.* (Pittsburgh, Pennsylvania)



tion of a vapour more strongly adsorbed than any of the components of the mixture. The displacement curve thus produced is illustrated in Fig. 1B. Each step corresponds to one component, and Claesson was able to demonstrate that, for a wide range of hydrocarbons, the step height was constant and characteristic for any particular component, and the step length was proportional to the amount of the component present in the mixture.

We have been applying Claesson's method to the analysis of the products obtained from experiments on gas kinetics, and, while the work is still very incomplete, it was felt that it might be valuable to indicate some of the main points that have so far emerged, if only to call attention to the simplicity and efficiency of the method. A full report of the work will be published later.

### Experimental

The apparatus (Fig. 2) consists of a device A for the production of a constant flow of nitrogen (carrier gas), means for passing the mixture on to the column of adsorbent B, and for the introduction of a constant concentration of displacer into the nitrogen stream C, the column D and the analyzer E.

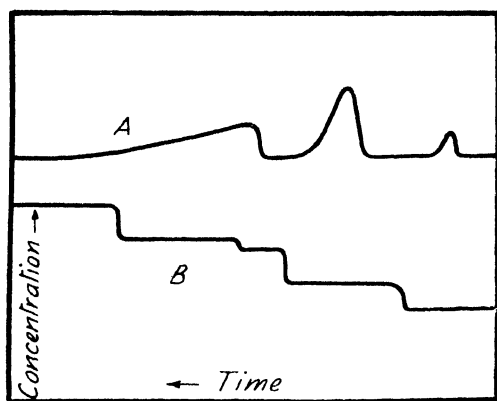


FIG. 1.—A. Elution analysis. B. Displacement analysis.

Nitrogen, obtained from a cylinder through a large buffering volume and a needle-valve, is passed through a simple compensating flowmeter, designed by Clark,<sup>4</sup> and illustrated in Fig. 3. In this device, the rate of gas flow, in the direction of the arrows, is fixed by the pressure of water BC across the capillary A. B is constant as long as the stream of bubbles is maintained, but C will alter with any change of the pressure inside the system, the difference in pressure between the system and the atmosphere being given by the height CD (+ EF). However, if the diameter of the inner tube is considerably less than that of the outer tube, the change in the internal pressure has normally only a negligible effect upon C. Any very marked alteration of pressure can be counteracted by adjusting the level of the water in the side tube EF.

The mixtures are conveniently brought on to the column by condensing them in a liquid-air trap, through which the nitrogen can subsequently be passed. The constant concentration of desorbent is obtained by passing the nitrogen through a saturator. We have used ethyl acetate in a bath at 0° C for most of the work hitherto. The column, a glass tube built in sections (see below), is surrounded by a jacket, which enables a vapour to be distilled around the column, so that experiments can be conducted at a series of temperatures. The column can readily be removed from the jacket for filling and cleaning.

<sup>4</sup> Clark (British Oxygen Co.) (private communication).

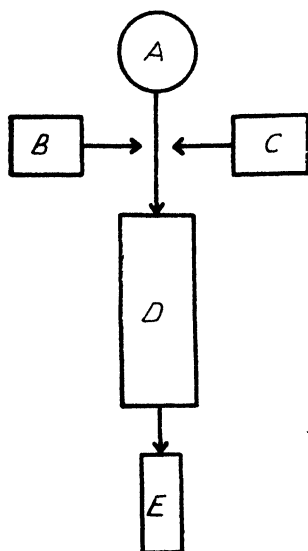


FIG. 2.—The apparatus.

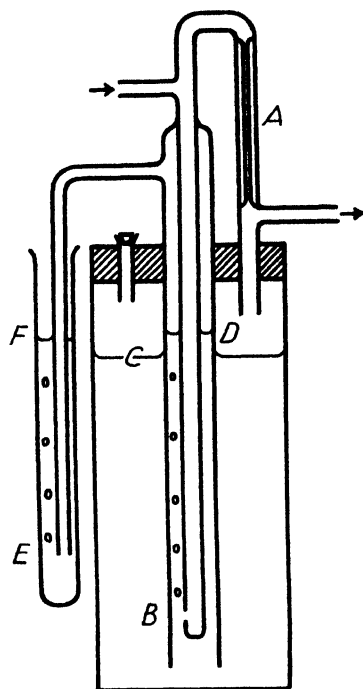


FIG. 3.—Compensating flowmeter (Clark).

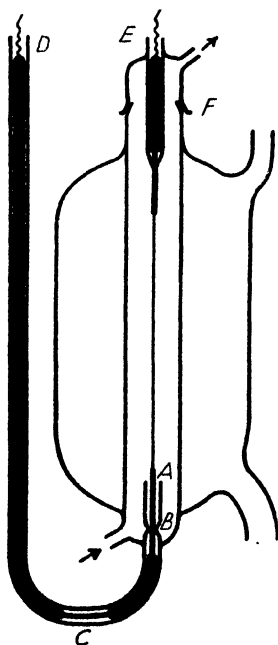
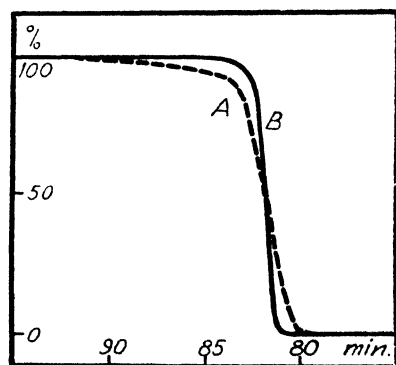


FIG. 4.—Thermal conductivity cell.

FIG. 5.—Fronts for ethyl acetate (in  $N_2$ , 80 ml./min.).

- A. Through 9 mm. diam. column (4 cm.<sup>3</sup> activated coal charcoal).
- B. Through the same followed by 2½ mm. column (½ cm.<sup>3</sup> charcoal).

A thermal conductivity cell constitutes the analyzer. This consists (Fig. 4) of a Pt wire (14 cm. long, 0.05 mm. diam.), heated electrically and passing centrally down a glass tube (1.0 cm. diam.), which is again surrounded by a jacket so that the cell can be operated at a variety of temperatures. The wire is held in position by a Pt weight A, which passes through a narrow constriction B into a Hg pool: C is a Pt wire sealed into the glass. Electrical contact is made at D and E. The ground joint F enables the wire to be removed and replaced with ease. The wire forms one arm of a Wheatstone bridge, the other three arms being similar wires kept in sealed tubes immersed in a thermostat. The voltage for the bridge and for heating the wire is supplied by a high-capacity 2-volt battery. A recorder (Ether Indicorder, full-scale deflection 1.8 mV, chart speed 1 in. in 5 min.) is placed across the centre of the bridge.

Provision has also been made for measuring the densities of the various components with a quartz buoyancy microbalance, previously used for atomic weight determinations. This, however, has proved unnecessary.

The accuracy of the analysis will be related to the degree of overlap between one component and the next, i.e., to the sharpness of the steps in the displacement curve. This has been found to depend upon a number of factors.

(i) PARTICLE SIZE.—In general, it is found that the sharpness of the step fronts is increased by diminishing the size of the particles of adsorbent and by increasing their homogeneity. In the work hitherto, we have found no marked advantage in reducing the size of the particles below B.S.S. 40 mesh, while smaller particles are inconvenient as they impede the flow of gas. Dust must be carefully avoided as it deposits on the tubes away from the rest of the adsorbent, and thus produces irregular and diffuse fronts.

(ii) FLOW-RATE.—The fronts are improved by slowing down the rate to about 50 ml./min., though below this rate the improved sharpness has been insufficient to merit further reduction. Flow-rates greater than 100 ml./min. tend to produce irregularities in the record of the analyzer.

(iii) COLUMN SIZE.—The sharpness of the fronts increases markedly with a lowering of tube diameter. For a given diameter the maximum separation is achieved in a remarkably small length of tube. In order to obtain sharp fronts, and yet to be able to work with fairly large quantities of material without resort to very long tubes, the column is built in sections, each section smaller than the one above and preceding it. The effect of this is shown in Fig. 5. The column in use at the time of writing is made of three 10 cm. lengths of tube, with internal diameters of 15 mm., 8 mm. and 2 mm. The adsorbent in each section is supported on a disc of wire-gauze. The same type of divided column has been used by Claesson for work with liquid chromatography.<sup>5</sup>

(iv) ADSORBENTS.—Activated charcoals have been used in most of the work hitherto. These are carefully sieved and dried at 120° C. Slightly improved fronts are obtained if the charcoal is first saturated with the desorbent (ethyl acetate), which is then removed by distilling, e.g., with boiling nitrobenzene round the column. For work with the more volatile gases (e.g., propane) a highly activated charcoal is recommended to prevent elution. An increased sensitivity is obtained by use of weakly adsorptive charcoals, owing to the increase in the specific length (step length per unit of component). This is particularly important with the higher molecular-weight components which have the smaller specific lengths. The same effect can, however, be obtained by using a more strongly adsorbed desorbent or a lower concentration of desorbent, by saturating the charcoal at an elevated temperature with a substance which will not be displaced or appreciably eluted, or by raising the temperature of the adsorbent.

(v) TEMPERATURE.—The effect of temperature is illustrated by the figures in Table I. The temperature of the thermal conductivity cell was the same (20° C) throughout. It will be noted that for each gas the product of step height and specific length is virtually constant, the thermal conductivity being approximately a linear function of the concentration.<sup>1</sup>

Sharp steps have been obtained with a range of organic vapours including hydrocarbons (saturated, unsaturated and aromatic), halogenated hydrocarbons,

<sup>5</sup> Claesson, *Arkiv. Kemi, Min. Geol. A*, 1947, **24**, No. 16.

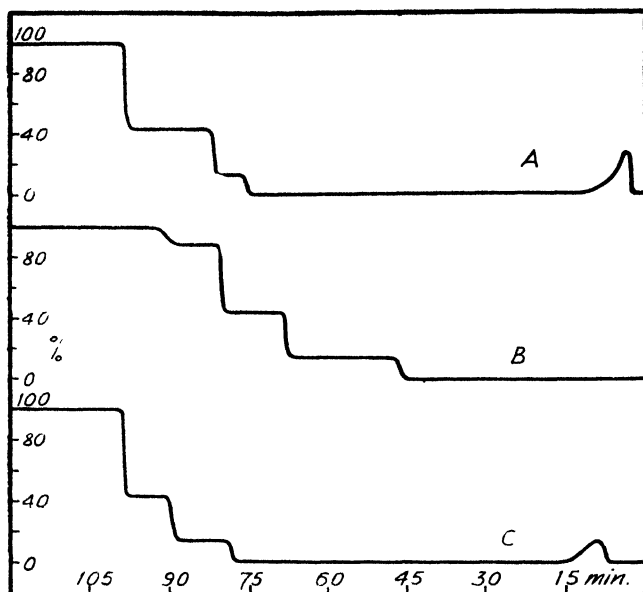


FIG. 6.—Displacement analyses of hydrocarbon mixtures.

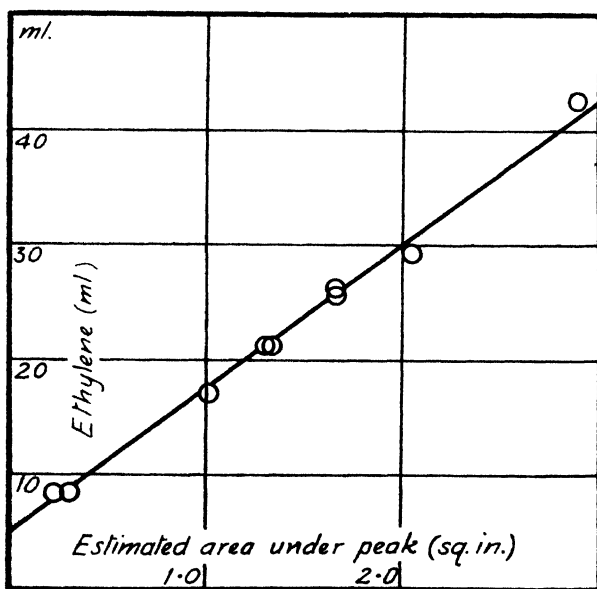
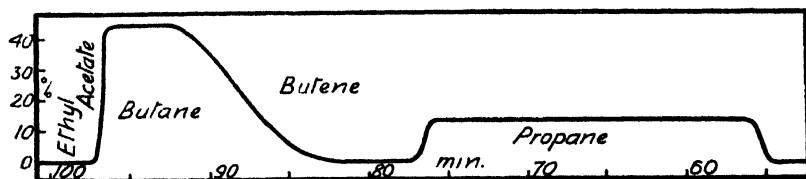


FIG. 7.

FIG. 8.—Separation of butane and butene.  
Adsorbent: Activated coal charcoal; flow-rate: 80 ml./min.

nitroparaffins, ethers, ketones, alcohols and esters. Some typical curves are reproduced in Fig. 6, and the corresponding analyses in Table II.

Ethylene is not displaced as a step, and the calculations have to be made by measuring the area under the peak. It is impossible to do this completely owing to the fact that the tail soon becomes indistinguishable from the record for  $N_2$ . However, if a plot is made of ml. ethylene (1 ml. = 0.00125 g.) against estimated area under peak, a straight line is obtained (Fig. 7), and from this the concentration of ethylene can be calculated. It will be noticed that the line does not pass through the origin, thus indicating the amount of material unobserved in the tail.

TABLE I

Gas	Propane		Butane	
Temperature (° C)	Step height (% of Ethyl Acetate)	Specific length (in./g.)	Step height (% of Ethyl Acetate)	Specific length (in./g.)
126.5	No step	—	12.0	135
57	7.0	232	30.5	53.0
20	17.5	95.5	44.0	36.3
6.5	23.5	71.7	47.0	34.4

Adsorbent: Dorsite (coconut charcoal); flow-rate: 80 ml./min.

TABLE II

Analysis	Component	Step height (% of Ethyl Acetate)	Found value (g.)	Correct value (g.)
A	Ethylene	—	0.0215	0.0213
	Propane	14.0	0.0038	0.0034
	n-Butane	43.5	0.0408	0.0413
B	Propane	14.0	0.0171	0.0167
	n-Butane	43.5	0.0334	0.0331
	n-Hexane	86.5	0.0265	0.0261
C	Ethylene	—	0.0108	0.0107
	Propane	13.5	0.0085	0.0083
	n-Butane	43.5	0.0246	0.0246

Adsorbent: 2 cm.<sup>3</sup> activated coconut charcoal (Sutcliffe, Speakman No. 208C); flow-rate: 40 ml./min.

The diffuse region between two components corresponds in almost all cases to flow period of about two minutes. This region is slightly increased between alcohols, a fact presumably to be related to their high association. The separation is particularly marked along an homologous series but with substances of similar boiling point the separation is less perfect and, moreover, is masked by the fact that two such substances will often have almost the same step height. It is, therefore, often convenient to combine the column separation with some other means. For example, butane and butene appear to come off together as one step, but if the gases, before entering the thermal conductivity cell, are passed through a tower, containing pumice soaked in conc. sulphuric acid, the butene is removed and a curve as in Fig. 8 is obtained. From this the percentage of each component is readily calculated. In this case the diffuse region corresponds to 10-min. flow period (i.e., 0.050 g. of the butane-butene mixture).

An alternative method can be devised using silica gel, for we have observed that, with the exception of ethylene, the unsaturated hydrocarbons are not displaced from silica gel by ethyl acetate, while the saturated hydrocarbons are removed readily.

A rather surprising feature of the method is that the accuracy with which the amount of one component is determined seems to be independent of the amounts of the other constituents present. The method is, therefore, very suitable for the estimation of a small percentage of one component in a relatively large amount of a mixture. Thus, in one series of experiments to determine the possible ether content of a sample of ethylene, no step corresponding to ether could be produced from 15 g. ethylene. 0.0714 g. ether was then mixed with the same amount of ethylene, and a step of 3.25 in. was obtained for the ether. The same quantity of ether, in the absence of the ethylene, gave a step length of 3.22 in. Fig. 9

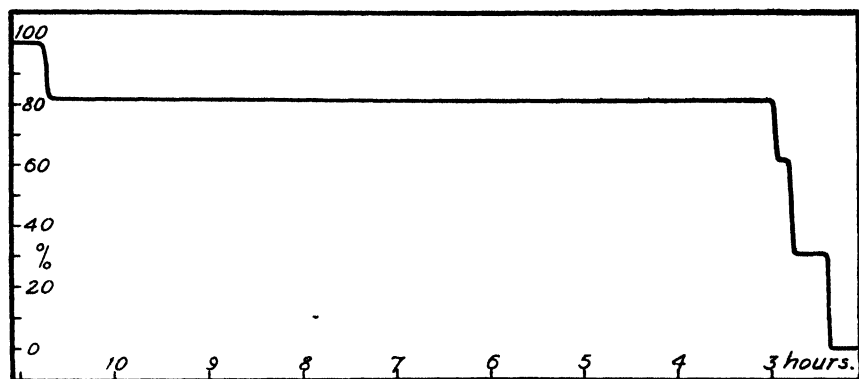


FIG. 9.—Analysis of residue from fractional distillation column.  
Adsorbent: 19 cm.<sup>3</sup> activated coal charcoal; flow-rate: 40 ml./min.

illustrates an analysis of a residue (approximately 1.5 ml.) from a fractional distillation column, in which 2.1 ( $\pm 0.1$ ) mole-% of  $C_6$  hydrocarbons and 8.6 ( $\pm 0.2$ ) mole-% of  $C_4$  hydrocarbons were separated from the main component, *n*-hexane.

Self-sharpening fronts and, in the case of elution, diffuse tails are to be expected, on the simple theory, for substances whose adsorption isotherms are convex towards the pressure axis. This has been generally observed. Conversely for substances, whose adsorption isotherms are concave towards the pressure axis, diffuse fronts and sharp tails should be observed. This effect is demonstrated in Fig. 10, which illustrates the elution of water from charcoal.

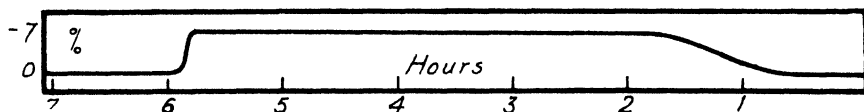


FIG. 10.—Elution of water from charcoal.

As a result of this behaviour water is not displaced as a single step by a desorbent such as ethyl acetate, but, if present in any but very small amounts, it produces a number of steps in company with other components. The length of the step, e.g., containing ethyl acetate and water, can be used as a measure of the water present, but in general much confusion is avoided if the mixture can be kept moisture-free.

The behaviour of aldehydes is curious. They are not displaced from charcoal by ethyl acetate or other similar desorbents. If, however, the charcoal is wet or the aldehyde contains water, then a normal step is produced. Further, while an aldehyde will tenaciously resist elution in  $N_2$ , it is readily removed by water vapour, or by the vapours of either methyl or ethyl alcohol. In each case the

aldehyde is desorbed in company with the vapour in question. The aldehyde, under such circumstances, can be used to displace other materials from the column.

The author wishes to thank Dr. S. Claesson, Prof. Sir Cyril Hinshelwood and Dr. B. Lambert for their valued advice and encouragement.

*Inorganic Chemistry Laboratory,  
Oxford.*

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## CHROMATOGRAPHY OF PETROLEUM HYDROCARBONS

BY W. M. SMIT

*Received 18th July, 1949*

A review of applications of the adsorptive percolation method using petroleum hydrocarbons is given. The development method with a series of developers is recommended if a more detailed separation of petroleum fractions is the aim. It is shown that, at least on silica gel, the hydrocarbons of a certain group arrange themselves during development according to their refractive indices. The results of adsorptive percolation through a silica gel column have been compared with a treatment in a thermal diffusion column, both applied to a completely hydrogenated lubricating oil-fraction. In the case studied, the result of adsorptive percolation is somewhat superior. The results of percolation of two other lubricating oils are discussed.

The use of the word chromatography in connection with hydrocarbons is mainly based on historical considerations. Other expressions less stressing the colour element for indicating the adsorptive separation method of Tswett, such as adsorptive percolation, might be more suitable. For, excepting aromatics which show fluorescence when exposed to ultra-violet light, hydrocarbons are colourless. Consequently, with hydrocarbons the process of adsorptive percolation is nearly always continued until all the constituents of the mixture have left the adsorption column and the effluents are analyzed separately, mainly by optical means (liquid chromatography). Besides this special feature, it is more important to realize that petroleum fractions are usually very complex mixtures of various groups of closely related substances. This accounts for the moderate success of adsorptive percolation when applied to petroleum fractions. Furthermore, the questions arising with adsorptive percolation of hydrocarbons are quite comparable with those arising with other compounds. These questions are: which method of adsorptive percolation is most suitable for the purpose aimed at, which adsorbent and which solvents may give the best results, and how is the separating efficiency as compared to other separation methods.

As to the methods, both theoretical and experimental work have now led to the general opinion that when a non-quantitative separation is required, the elution method (displacement method) may give good results. When incomplete separation into two fractions is sufficient, or when not too complex mixtures have to be analyzed, the continuous introduction method (frontal analysis) may be suitable. However, when complex mixtures have to be investigated or complete separation is aimed at the development method has to be chosen if its use is possible.

Concerning the adsorbents and solvents to be used, no general rules have so far been found. Experiences of other investigators or the trial and error method have to be referred to. Therefore, a review of the literature on

adsorptive percolation of hydrocarbons will be given first. Following this, some experiences with petroleum fractions will be mentioned and, as a further contribution to the question of the efficiency for a special case, a comparison will be made between adsorptive percolation and thermal diffusion. Finally, the applicability of adsorptive percolation to petroleum fractions and the possibilities connected therewith will be summarized. The following survey of the literature has been made according to the methods of percolation used.

The continuous introduction method is applied on a technical scale to remove coloured substances from certain petroleum distillates. The adsorbent used is bauxite or active clay.

Hirschler and Amon<sup>1</sup> showed that continuous introduction of the sample followed by elution with methyl alcohol constitutes a useful purification method for saturated hydrocarbons which are contaminated with homologues. Silica gel and charcoal are suitable adsorbents, whereas alumina is far less effective. A large number of data concerning the adsorption of binary systems of saturated hydrocarbons up to  $C_{12}$  is given. The authors show that the method has its limitations when so-called S-type adsorption isotherms occur.

For analytical purposes Mair and White<sup>2</sup> applied the elution method to a mixture of hydrocarbons boiling between  $80^{\circ}$  and  $175^{\circ}$  C. Using silica gel as adsorbent and water as eluent (displacer) the mixture could be separated into an aromatic and a non-aromatic fraction. Willingham<sup>3</sup> obtained the same result with a mixture of higher boiling-point hydrocarbons. Mair<sup>4</sup> using methanol as eluant analyzed a synthetic mixture of toluene, cyclohexene, 2 : 2 : 4-trimethylpentene and 2 : 2 : 4-trimethylpentane by percolation through silica gel. Toluene and 2 : 2 : 4-trimethylpentane were recovered in an approximately pure state. Cyclohexene and 2 : 2 : 4-trimethylpentene could not be separated from each other. The elution method is also applied to gaseous hydrocarbons up to heptane. An ingenious apparatus has been constructed by Turner.<sup>5</sup> The adsorption column is filled with pellets of activated carbon. Elution is accomplished by a combined action of heat and mercury. The effluent gases are analyzed by means of heat conductivity cells. Hydrocarbons of different molecular weight may be separated but difficulties are encountered with the separation of isomers. A similar apparatus has been constructed by Tiselius and Claesson.<sup>6</sup>

With the development method the effluents are always diluted by some solvent. Therefore, the method is usually restricted to hydrocarbons of low volatility. Using alumina as adsorbent and benzene as developing solvent, Winterstein and Schön<sup>7</sup> separated polycyclic aromatics. Mair and Forziati<sup>8</sup> carried out a quantitatively controlled adsorptive percolation of a mixture of seventeen hydrocarbons. The mixture was separated into an aromatic and a non-aromatic fraction and the composition found tallied within 1 % with the true composition. Silica gel was used as adsorbent and propane, butane or pentane were the developing solvents. Nederbragt and de Jong<sup>9</sup> obtained complete separation of a mixture of tetracosane and di-sec-butyldecaline using an active earth (Floridine 267) as adsorbent and pentane as developer. With another kind of active earth (Floridine XXF) even the

<sup>1</sup> Hirschler and Amon, *Ind. Eng. Chem.*, 1947, **39**, 1585.

<sup>2</sup> Mair and White, *J. Res. Nat. Bur. Stand.*, 1935, **15**, 51.

<sup>3</sup> Willingham, *J. Res. Nat. Bur. Stand.*, 1939, **22**, 321.

<sup>4</sup> Mair, *J. Res. Nat. Bur. Stand.*, 1945, **34**, 435.

<sup>5</sup> Turner, *Petr. Ref.*, 1943, **22**, 140.

<sup>6</sup> Claesson, *Ann. N.Y. Acad. Sci.*, 1948, **49**, 183.

<sup>7</sup> Winterstein and Schön, *Z. physiol. Chem.*, 1934, **230**, 139, 146.

<sup>8</sup> Mair and Forziati, *J. Res. Nat. Bur. Stand.*, 1944, **32**, 151.

<sup>9</sup> Nederbragt and de Jong, *Rec. trav. chim.*, 1946, **65**, 831.



separation of two naphthenes, viz., octadecylcyclohexane and di-*sec*-butyldecaline, proved to be possible.

Recently the author<sup>10</sup> showed that introduction of a suitable series of developers may be very useful when a complex mixture of hydrocarbons has to be separated. Out of a mixture of five hydrocarbons, viz., hexadecane, hexadecene, dodecylbenzene,  $\alpha$ -propylnaphthalene and 1:2-diphenylpropane, all components could be re-collected in their original pure state. Only between the two components last mentioned an intermediate fraction was found. The other components were re-collected quantitatively in a pure state. The adsorbent used was silica gel and the developers used subsequently were pentane, carbon tetrachloride and chloroform. It proved to be possible to calculate the result to be expected from figures obtained on percolation of the pure substances. The result calculated and the result actually found agreed within the experimental error. For further details the original paper should be referred to.

### Experimental

**Petroleum Fractions.**—It would have been of interest to extend the work with pure hydrocarbons previously mentioned to still more complex mixtures of known composition. For temporary lack of suitable pure

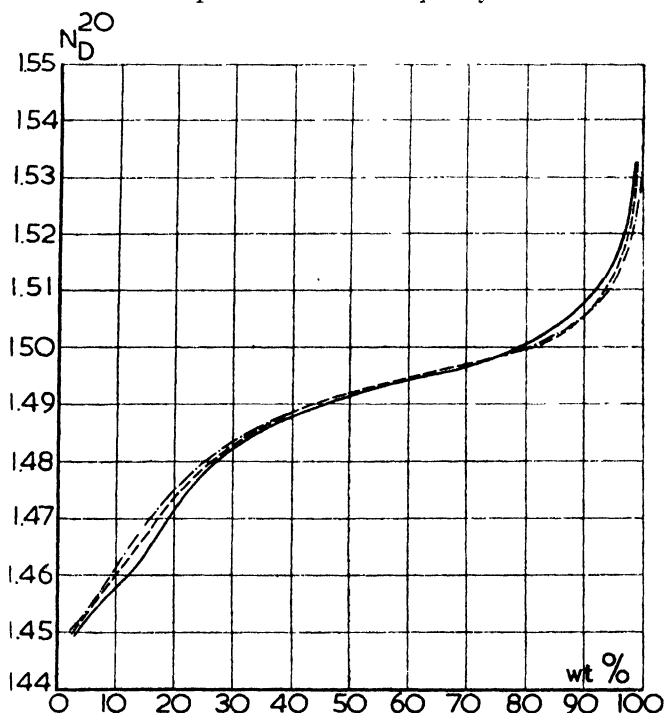


FIG. 1. Percolated sample: completely hydrogenated lubricating oil fraction. Absorbent: silica gel.

Column int. diam. (cm.)	...	...	...	1.45	2.9	5.8
Length of packing (cm.)	...	...	...	310	302	301
Weight of packing (g.)	...	...	...	336	1345	5242
Density of packing (g./ml.)	...	...	...	0.66	0.67	0.66
Sample weight (g.)	...	...	...	3.754	15.525	60.145
Line	...	...	...	Drawn	Broken	Dash-dot

<sup>10</sup> Smit, *Anal. chim. Acta*, 1948, 2, 671

hydrocarbons some lubricating oil distillation fractions have now been investigated using the same method. This method may be restated briefly. A glass \* adsorption tube is mechanically filled with silica gel (Davison, through 200 mesh). The length of the columns used is about 300 cm. and the cross-sectional area is taken proportional to the weight of sample to be handled. As Fig. 1 shows, the separating efficiency is not affected by the diameter if the length of the column is kept constant and the sample size is taken proportional to the square of the diameter of the column. The weight ratio of sample to silica gel is about 1/100. Before introduction of the sample the column is wetted with the first developer to be used so as to diminish loss of material caused by irreversible adsorption. By doing so losses would be reduced from 4 % without previous wetting to 1 % with pre-wetting. The sample is previously diluted by about ten times its volume of the first developer. Then the sample

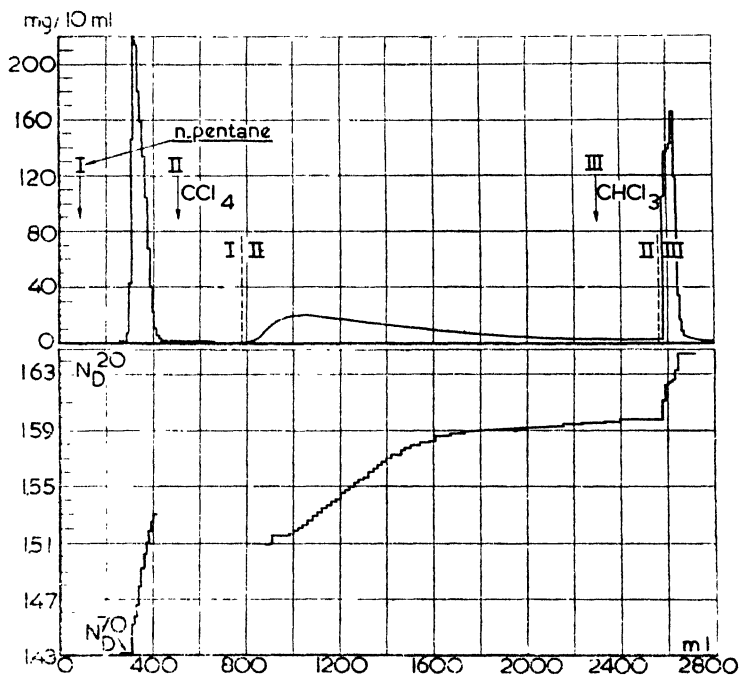


FIG. 2.—Result of adsorptive percolation of sample A. (Lubricating oil fraction containing hardly any paraffins.)

is introduced into the column. Subsequently the first developer is forced through the column so that the linear speed of the liquid front does not exceed 2 cm./min. Samples of appropriate volume are collected at the bottom of the column. The solvent is evaporated, the residue is weighed and its refractive index is measured. Introduction of the same developer is continued until evidence has been obtained that the effluent leaves no perceptible residue after evaporation of the solvent. Then the next developer to be used is introduced into the column. The same process is repeated, after which a third developer is introduced, etc. Thus far three developers have been used, viz., pentane, carbon tetrachloride and chloroform. After chloroform has become ineffective the components still retained in the column are eluted by means of ether or acetone. For the determination of the right moment of changing the developer and the sequence of the developers the experiments with pure hydrocarbons<sup>10</sup> have been of great help.

\* It may be mentioned here that some oils adsorbed on silica gel may give rise to the formation of coloured products when exposed to daylight.

The results of the percolation of two oil fractions of different origin have been represented (qualitatively) by Fig. 2 and 3. Sample A (Fig. 2) is a lubricating oil fraction which according to ring analysis <sup>11</sup> (the original method has been modified and will be published shortly) contained naphthenes and aromatics and no paraffins. However, the first percolation fractions of this sample solidified at room temperature. Moreover, the refractive index (measured, by way of exception, at 70° C) was 1.431. It was concluded that the very first fractions consisted of paraffins. Thus the presence of a few tenths per cent. of paraffins can be detected in a mixture mainly consisting of naphthenes and aromatics. The following pentane fractions had refractive indices increasing from about

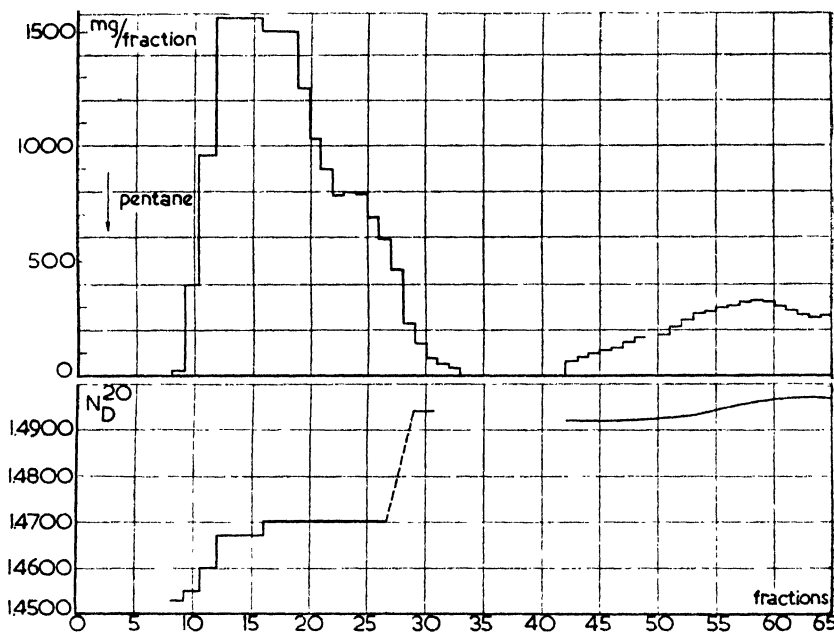


FIG. 3.—Result of adsorptive percolation of sample B. (Low boiling-point lubricating oil fraction.)

1.453 to 1.530, suggesting that the main part of the naphthenes was of polycyclic nature.<sup>12</sup> The pentane fractions when examined by an ultra-violet spectrometer showed no trace of aromatics. The fractions obtained with carbon tetrachloride had refractive indices increasing from 1.510 to 1.597. The chromatogram revealed that only a small amount of monocyclic aromatics was present. Indications have been obtained that the carbon tetrachloride fraction contained mainly dicyclic and perhaps some polycyclic aromatics. The refractive indices of the chloroform fractions increased from 1.611 to 1.644. These fractions contained the polycyclic aromatics. After chloroform had ceased to be effective, about 10 % of the oil was still left in the column. This last part was recovered by introducing ether into the column. After evaporation of the solvent a sticky brown mass was obtained which showed a remarkably high sulphur content. Thus it was clear that sulphur compounds and presumably other non-hydrocarbons were accumulated in the last percolation fractions.

As to sample B (Fig. 3), which had a lower mean molecular weight than sample A, only the pentane part of the chromatogram has been represented.

<sup>11</sup> Vlughter, Waterman and van Westen, *J. Inst. Petr. Techn.*, 1935, **21**, 661, 701.

<sup>12</sup> Smittenberg and Mulder, *Rec. trav. chim.*, 1948, **67**, 813, 826.

The remaining part showed no peculiar differences when compared with sample A. According to ring analysis, sample B consisted of paraffins, naphthenes and aromatics. As is seen in Fig. 3, there is a distinct gap in the pentane fractions. First a series of fractions was obtained, the refractive indices of which increase continuously up to 1.470. Then several fractions of pentane were collected leaving no residue after evaporation of the solvent. However, the next pentane fractions after evaporation of the solvent left residues the refractive indices of which start at 1.491 and rise slowly up to 1.500. So a separation between mono and dicyclic naphthenes was obtained here. To confirm this the pentane fractions were examined with an ultra-violet spectrometer. No aromatics could be detected.

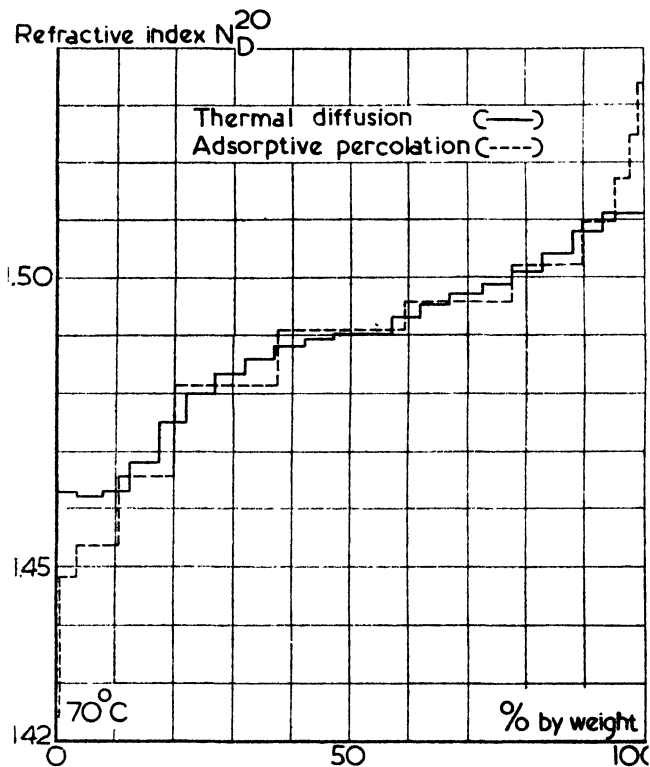


FIG. 4.—Comparison of adsorptive percolation and thermal diffusion for the special case of a completely hydrogenated lubricating oil fraction of the same type as sample A.

These two examples of adsorptive percolation show that this method not only permits determination of the ratio between aromatic and non-aromatic compounds, but also gives valuable information about the constituents within one of these groups. Thus adsorptive percolation may be a useful means of characterization. Yet it seemed useful to ascertain whether after a first separation in two groups other physical separation methods might give even more detailed separation between the constituents of one group. Thus, thermal diffusion was compared with adsorptive percolation. Preliminary experiments had shown that thermal diffusion gives no specific separation between aromatics and non-aromatics. Therefore a distillation fraction of oil A (which contains hardly any paraffins) was hydrogenated completely, thus giving a mixture consisting of naphthenes only. One part of the hydrogenated oil was submitted to adsorptive percolation in a silica gel column of length 300 cm. using pentane as developing solvent. The other part was submitted to thermal diffusion.

The thermal diffusion column has a length of 180 cm. It consists of two concentric brass cylinders leaving an annular slit of 0.25 mm. thickness. The surface of the outer cylinder is cooled with tap water. The inner tube is heated by steam, thus causing an effective temperature difference of about 50° C across the slit.

One thermal diffusion run lasts about 240 hr. and requires 5 tons (weight) of steam. The size of the sample is about 40 ml. The adsorption column has an inner diameter of 1.5 cm. The percolation lasts about 8 hr. About 700 ml. pentane is used which may be recovered. The size of the sample is 4 g. If the sample had been of the same amount as used in the thermal diffusion experiments, the diameter of the adsorption column should have been about 4.5 cm. and about 7 l. pentane would have been necessary, whereas the percolation time would have remained the same, viz., 8 hr. The results of both methods are represented graphically in Fig. 4, whence it may be concluded that the separation obtained by adsorptive percolation is in this case somewhat superior to that by thermal diffusion.

### Discussion

According to the literature and our own experience it may be stated that quantitative separation of petroleum fractions into an aromatic and a non-aromatic part is hardly a problem nowadays. The elution (displacement) method may give good results when silica gel is used as adsorbent and alcohol, acetone or ether are used as eluants. Water is less suitable. The results may be greatly improved, especially when viscous oils are dealt with by previous dilution of the sample with a mixture of pentane and some other solvent like carbon tetrachloride, chloroform or benzene, which is more strongly adsorbed. A weight ratio of gel/sample above 10/1 is found useful. This method may be also used to accumulate non-hydrocarbons present in oil fractions.

For further separation within the groups of saturated and non-saturated hydrocarbons the developer method is more suitable. This becomes the clearer if the experiments of Hirschler and Amon<sup>1</sup> are taken into account. As yet, percolation through silica gel has shown the possibility of separation between mono and polycyclic compounds, both saturated and non-saturated. However, complete separation has not been established with certainty. As a general feature of adsorptive percolation through silica gel using the developer method, it may be mentioned that the hydrocarbons are arranged according to their refractive indices.

Though silica gel as adsorbent and pentane as developer constitute a good combination to resolve a mixture of saturated compounds, some active earths may presumably give better separation between paraffins and naphthenes.

From separate experiments<sup>10</sup> and the results mentioned in this paper, it may be concluded that carbon tetrachloride has a rather weak developing power, whereas chloroform is adsorbed somewhat too strongly to effect fair separations in the aromatic part, at least when silica gel is the adsorbent. Experience with mixtures of these two solvents has as yet not been very successful. Other solvents have been tried and C<sub>6</sub> olefins might be very suitable but these are polymerized on silica gel. Better results may perhaps be obtained if the aromatic part is re-percolated through alumina as seem to be borne out by the experiments of Winterstein.<sup>7</sup>

When comparing the result of adsorptive percolation with other separation methods applicable in petroleum chemistry, the conclusion arrived at depends on the result desired and the range of molecular weights covered by the constituents of the sample. Up to C<sub>6</sub>, low-temperature distillation has several advantages if nearly complete separation is required. From C<sub>6</sub> up to about C<sub>16</sub>, a combination of distillation and adsorptive percolation may

give good results. But for heavier compounds, distillation permits only a rough separation according to molecular weight, whereas adsorptive percolation is far more selective as to the nature of the compounds. Moreover, with distillation partial decomposition of higher boiling-point compounds can hardly be avoided. Fractional crystallization at low temperatures may be of help in this region but is also less selective. Thermal diffusion is to some extent comparable with adsorptive percolation, except in the separation between saturated and non-saturated compounds.

In conclusion, for petroleum hydrocarbons it may be stated that adsorptive percolation constitutes a very useful, additional separation method, especially in the range above  $C_{16}$ . However, the investigation of its possibilities and limitations is far from complete.

Acknowledgment is due to the management of the Bataafsche Petroleum Maatschappij for their permission to publish this work and to the author's colleague Mr. Kramers for permission to make use of results of his experiments on thermal diffusion.

*Koninklyke/Shell-Laboratorium,  
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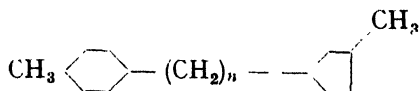
## CHROMATOGRAPHIC FRACTIONATION OF BLACK OILS

BY A. S. C. LAWRENCE AND D. BARBY

*Received 1st July, 1949*





Preliminary attempts at chromatographic fractionation of black oils have given indications that separation into hydrocarbon types is achieved to a great extent and that the method should be of great value in the study of petroleum. The resinous components most strongly adsorbed on alumina and coke contain significant amounts of oxygen.

Very little is known of black oils; they are treated as by-products. They were regarded first as mixtures of all possible isomers of paraffins, and when it was recognized that the three main classes of hydrocarbons, viz., paraffins, naphthenes (i.e., cyclo-paraffins) and aromatic hydrocarbons, were all present, the idea arose that the higher oils could not be assessed on any basis of these types because of the existence of mixed molecules. In particular, on this chemical basis it is suggested that all asphalts are different and that there is such an astronomical number of possible components that any examination would be a waste of time. This is true only if the object is to separate such mixtures into their individual chemical components. In view of the evidence and as a result of previous work by one of the writers (A. S. C. L.), it was considered that there is not unlimited mixing of the three types of hydrocarbon in oils of high molecular weight of the type:



although alkyl naphthenes with short alkyl groups are certainly common in petrol fractions. It was further considered that the more serious factor

to be considered was that we have in the simplest case, where there is no mixing of type, three homologous families, viz. :

PARAFFINS		CYCLO-PARAFFINS		AROMATICS
Methane .. ..		E.g., 	..	Benzene + 
Ethane .. ..		 ..		Naphthalene
Propane .. ..		 ..		Anthracene
Butane and isomers..				

In very large molecules a small amount of alkyl substitution or linking of rings will not detract much from the ideal type.

With high molecular weight compounds, specificity of solvent action will increase, but even if the higher members of one class are insoluble in a chosen solvent in which another class is soluble, the lower members of the insoluble class will become soluble. It is necessary therefore to carry out 'horizontal' as well as 'vertical' separation. This is important for another reason. The highest molecular weight material in asphalts and black oils, the so-called 'hard asphalt,' is a very powerful absorbent of the lower molecular weight materials which in turn absorb still lower components.

### Experimental and Results

Following a number of preliminary experiments, an analysis was made of a Venezuelan fuel oil. The 'horizontal' separation was achieved by distillation in a good vacuum by which four volatile fractions and a residuum were obtained. Each was then fractionated chromatographically by the method of successive elutriation. Finally the whole undistilled oil was fractionated chromatographically for comparison. Powdered coke was used as absorbent at first, but alumina was found to give equally good results and this was used on account of the advantage of its colour.

TABLE I

Fraction No.	Boiling Range °C	% of Oil
54	140-200	18.45
55	200-230	11.06
56	230-290	11.55
57	290-340	11.2
58	Residuum	46.4
117	Whole Oil	—

A battery of tubes was erected each 18 in. long and 1½ in. diam. These discharged into Buchner flasks connected to a filter pump and the solution of the oils was sucked through the columns. This method has the great advantages that the elutriate can be passed from the first column to the second (and the process continued as required), and that where elutriation is very rapid with subsequent solvents, the elutriate can be diluted to 2 % before running through the second and subsequent tubes.

The oils were made up to 2 % solution in hexane and sucked through alumina columns: a colourless solution containing white oil was obtained ( $\alpha$  fraction). The alumina was then elutriated with cyclo-hexane which carries through a pale yellow oil with blue fluorescence ( $\beta$  fraction). This is followed by toluene

TABLE II

Fraction	$\alpha$	$\beta$	$\gamma$	$\delta_1$	$\delta_2$	$\epsilon$	$\zeta$	$\omega$
54								
% .. ..	86.0	2.22	4.68	Trace	—	—	—	—
$n_D$ .. ..	1.4830	1.5664	1.6246	—	—	—	—	—
Dispersion .. ..	0.0108	0.0202	0.0312	—	—	—	—	—
Colour .. ..	None	None	Brown	Brown	—	—	—	—
			Green	None	—	—	—	—
Fluorescence ..	None	None	Blue	—	—	—	—	—
55								
% .. ..	72.7	5.06	17.2	2.46	2.52	—	—	—
$n_D$ .. ..	1.4952	1.5550	1.6166	1.5953	—	—	—	—
Dispersion .. ..	0.0099	0.0174	0.0275	0.0237	—	—	—	—
Colour .. ..	None	Pale Yellow	Orange Yellow	Yellow	Brown	—	—	—
Fluorescence ..	—	—	—	—	—	—	—	—
56								
% .. ..	61.6	2.94	22.2	3.91	2.28	—	—	—
$n_D$ .. ..	1.4995	1.5331	1.6095	—	—	—	—	—
Dispersion .. ..	0.0101	0.0135	0.0259	—	—	—	—	—
Colour .. ..	Pale Yellow	Pale Yellow	Red	Brown	Brown	—	—	—
Fluorescence ..	Blue	Blue	Green	Green	None	—	—	—
57								
% .. ..	35.3	4.5	43.4	7.66	7.00	—	—	0.834
$n_D$ .. ..	1.4910	1.4850	1.5590	1.5730	—	—	—	—
Dispersion .. ..	0.0081	0.0079	—	0.0174	—	—	—	—
Colour .. ..	None	Pale Yellow	Red	Red	Red	—	—	Black
Fluorescence ..	None	Blue	Green	Green	None	—	—	None
58								
% .. ..	21.8	2.6	35.4	0.8	10.6	1.8	Trace	23.6
Colour .. ..	*	*	Dark Red	Dark Red	Dark Red	Black	Black	Black
117 (Whole Oil)								
% .. ..	40.0	11.5	23.0	5.0	7.0	1.5	0.5	7.5
$n_D$ .. ..	1.5002	1.5112	—	—	—	—	—	—
Dispersion .. ..	0.0108	0.0097	—	—	—	—	—	—
Colour .. ..	None	Pale Yellow	Red	Brown	Brown	Black	Black	Black
Fluorescence ..	None	None	Green	None	None	None	None	None

\* See text.

which removes an orange-brown oil with bright-green fluorescence ( $\gamma$  fraction). When the toluene elutriate runs colourless, chloroform is used: this always gives two fractions, one easily washed out,  $\delta_1$ , while a sharp brown band moves down the column slowly,  $\delta_2$ . The first extraction was always complete before the second elutriate reached the bottom. With higher fractions and residua, there still remained material adsorbed on the alumina. This was elutriated successively with carbon disulphide and pyridine both of which gave rapid and sharp separations ( $\epsilon$  and  $\zeta$  fractions). The chloroform,  $CS_2$  and pyridine fractions were dark resins without fluorescence. Any material insoluble in the hexane was named the  $\omega$  fraction.

Table I shows the fractions obtained by the vacuum distillation of the oil while Table II gives the results of the chromatographic fractionation.

Fig. 1 shows the composition of the oil together with the chromatographic fractionation of the whole undistilled oil. The total  $\alpha$  fraction for the fractionated oil is too large because it was not realized in dealing with the two most volatile fractions how difficult is the separation of the  $\alpha$  and  $\beta$  fractions, so that these



two  $\alpha$ 's are slightly yellow due to some  $\beta$  in them. The colour of the higher fractions of the whole oil shows that separation is considerably less sharp, but the greatest difference is in the  $\omega$  fractions. It might be suggested that distillation has caused polymerization and increased the  $\omega$  fraction in the distillation residuum, but we do not accept this view. No cracking took place and the  $\epsilon$  and  $\zeta$  fractions of the whole oil contained much black suspended matter which was absent from the corresponding distillate residuum fractions.

Attempts at chromatographic separation at 10 % concentration in hexane failed to give the sharp separation obtained at 2 %, judged by colour.

#### VENEZUELAN FUEL OIL

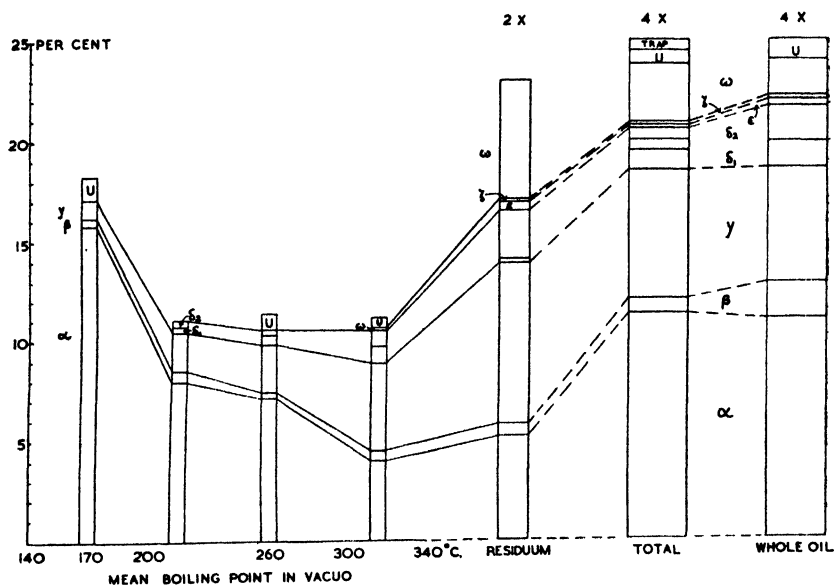


FIG. 1.

The residuum shows well the dual separation, the horizontal and the vertical. The hexane elutriate was evaporated in five successive portions and the residues showed progressive increase of viscosity and refractive index. The  $\beta$  fraction was recovered in three fractions whose viscosity also increased greatly. They were too viscous for determination of refractive index, but their flow was Newtonian as was that of the  $\alpha$  fractions.

The fuller characterization of the fractions is being undertaken, but it is considered that the sharpness of the separation as judged by colour and supported by the evidence of refractive index justifies communication of these early results. A residual asphalt from Persian fuel oil gave results very similar to that from the Venezuelan oil. Perhaps the most remarkable feature of this work is the consistency of fractionation found. It is felt that the method should be of the greatest value in studying changes occurring in refinery treatment of oils. It also provides a method for determination of wax in residua which is superior to any method so far proposed. The wax is all found in the  $\alpha$  fraction from which it is removed, after evaporation of the hexane, by methyl ethyl ketone. The wax so recovered from the vacuum distillation residuum of a Persian fuel oil had a melting point considerably lower than those of the distillate waxes.

This work forms part of certain researches being carried out for Engineer-in-Chief, Admiralty.

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# CHROMATOGRAPHY OF THE CARBOXYLIC ACIDS

BY HAROLD G. CASSIDY AND F. H. MAX NESTLER<sup>1</sup>

Received 13th July, 1949.

The principles and chief methods of application of chromatography as used in the separation of fatty acids are discussed. The applications of elution analysis, frontal analysis, displacement analysis and partition chromatography are reviewed, with attention to their advantages and disadvantages.

The separation of mixtures of homologous carboxylic acids into their components is difficult. The difficulty is greater as the amount of mixture available is smaller, as the mixture is more complex, as the components are more closely related and as the analytical requirements are more stringent. There is at present no way to separate small amounts of fatty acids in mixtures with five or more closely related components with a yield of 99 % of each acid. There is much evidence that this is not an impossible task, however, and excellent separations approaching this acuity have been reported. In this respect chromatography may be used in two ways: as an analytical tool which gives information about *how many*, and which, acids are present in the mixture, and *how much* of each is there; and as a preparative tool, which by the nature of its action goes a step farther and yields all the components of the mixture in pure form.

The second use of chromatography, that for the isolation of the pure fatty acids, was the earliest and is still the most important application. There are situations, as will be pointed out below, in which the first-mentioned use of chromatography is to be chosen. Earlier investigations of the chromatographic separation of carboxylic acids (fatty acids in these cases) were made by Kondo,<sup>2</sup> Manunta,<sup>3</sup> Kaufmann,<sup>4</sup> Cassidy<sup>5</sup> and Caliri.<sup>6</sup> These investigations showed that fatty acids could be separated on a variety of adsorbents such as alumina, frankonite, silica gel and charcoal. On the whole the separations were only fair. Kaufmann established the versatility of the method in extensive applications to saturated and unsaturated fatty acids and glycerides; Cassidy examined the relation between the static isotherms given by individual and mixed fatty acids and their chromatographic behaviour. The methods, though promising, were often tedious.

**General Aspects of the Chromatographic Method as Applied to the Separation of Fatty Acids.**—Chromatography is one of the separation methods based on a distribution between phases applied in a counter-current manner.<sup>7</sup> The distribution involved in chromatography is that between a bulk phase and an interfacial phase. In partition chromatography the distribution is in some instances between two more or less bulk phases and in others a rather complicated distribution involving bulk and interfacial phases. In any case the method may be compared with others which involve distribution

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<sup>2</sup> Kondo, *Pharm. Soc. Japan, Trans.*, 1937, **57**, 218

<sup>3</sup> Manunta, *Helv. chim. Acta*, 1939, **22**, 1156.

<sup>4</sup> Kaufmann, *Fette Seifen*, 1939, **46**, 268, and later papers; *Angew. Chem.*, 1940, **53**, 98.

<sup>5</sup> Cassidy, *Dissertation* (Yale, 1939); *J. Amer. Chem. Soc.*, 1940, **62**, 3073, 3076; *ibid.*, 1941, **63**, 2735.

<sup>6</sup> Caliri, *Dissertation* (Fribourg, 1939).

<sup>7</sup> Cassidy (submitted for publication).

between phases.<sup>8</sup> The fundamental fact upon which all of these distribution separations is based is that described by the principle of homology. In the case of adsorption this principle is given restricted expression in Traube's rule: that the extent of adsorption under certain conditions increases regularly along an homologous series. This rule holds well (qualitatively) for adsorption from bulk solution to an air-solution interface, and in many cases also for adsorption to a solid-solution interface. In other cases a reversal of this behaviour is observed, and where the adsorption is more or less dependent on the functional group of the molecule, it does not hold at all.

The rule of Traube may be generalized somewhat: the extent of adsorption at a given interface usually changes in a regular manner from one homologue to another. The chromatographic separation of fatty acids, or of any other substance, rests in part upon this observed behaviour, and where a series of well-separated isotherms are observed for a group of homologues then chromatographic separation may be feasible. Linner and Gortner<sup>9</sup> have published data on the adsorption of carboxylic acids from water onto Norit charcoal. The isotherms of the acids from formic to caproic are well separated, and it should be feasible to separate these acids using this adsorbent, unless other factors prevent (see below). The same may be said for the acids *n*- and isobutyric and *n*- and isovaleric; and among substituted acids, acetic and glycolic; acetic, glyoxylic and oxalic; caproic, adipic and citric; succinic, malic and tartaric; fumaric, succinic and maleic. In other cases the possibility of a separation is not so promising. Thus with the dicarboxylic acids from malonic to adipic the curves cross, though oxalic is well separated from the others; the propionic and pyruvic curves are fairly close together but well separated from glyceric and lactic which are close together; citraconic and itaconic are close together but mesaconic is better adsorbed, and the isotherm well separated from those of the other two, methyl succinic being less well adsorbed than the other three.

While well-separated isotherms imply chromatographic separability this cannot be the whole story, for otherwise separations on alumina, which in equilibrium experiments adsorbs many fatty acids to the same extent, would not be possible. But separations on alumina have been carried out, as has been mentioned above. Another factor which must be present lies in the non-equilibrium nature of the chromatographic adsorption as it is usually carried out. Even though two substances may at equilibrium be adsorbed to the same extent, yet if they are adsorbed at different rates it is to be expected that some separation could be achieved if the chromatography were carried out not too slowly. Thus mixtures which under equilibrium conditions show no preferential adsorption of one component over the other (corresponding, thus, to azeotropes and eutectics in the vapour-liquid and solution-solid distributions) should be separable by non-equilibrium chromatography just as azeotropes and eutectics may be separated, or broken, by non-equilibrium evaporation and non-equilibrium crystallization respectively. Such mixtures have been found in adsorption from some systems in which the adsorptions of two substances have been studied over the entire concentration range.<sup>10</sup> It is to be expected that rates of adsorption would change in a regular manner over an homologous series.

<sup>8</sup> Randall and Longtin, *Ind. Eng. Chem.*, 1938, **30**, 1063. Cassidy, *J. Chem. Educ.*, 1946, **23**, 427; *Ann. N.Y. Acad. Sci.*, 1948, **49**, 143. Thiele, *Ind. Eng. Chem.*, 1946, **38**, 646. Benedict, *Chem. Eng. Proc.*, 1947, **43**, 41.

<sup>9</sup> Linner and Gortner, *J. Physic. Chem.*, 1935, **39**, 35.

<sup>10</sup> Very many of these cases are known. For some recent examples see Bartell and Lloyd, *J. Amer. Chem. Soc.*, 1938, **60**, 2120, and Rao and Jatkar, *Quart. J. Indian Inst. Sci.*, 1942, **5**, 73, 80, 87, 91, 98. The correspondence to azeotropes and eutectics has been pointed out.<sup>11</sup>

<sup>11</sup> Cassidy, *Federation Proc.*, 1948, **7**, 464.

Chromatography is essentially a method of using the adsorption distribution in a differential counter-current manner.<sup>7</sup> The procedures which are now commonly used have been given descriptive names by Tiselius and his co-workers, who invented some of them.<sup>12</sup> These procedures are elution analysis, frontal analysis, displacement analysis. An additional method of a somewhat different sort has been invented and developed by Martin and Synge<sup>13</sup> and their co-workers: partition chromatography. The application of these methods and procedures, together with increased understanding of the problems, has improved the separations of carboxylic acids over those achieved in the earlier work referred to above.

**Elution Analysis.**—The earlier work on the separation of fatty acids cited above was carried out largely by elution analysis, the liquid chromatogram procedure.<sup>14</sup> Ideally, in elution analysis the zones<sup>15</sup> of separated fatty acids are made to march from the column in an ordered array, and are recognized by titration or by some other method. As stated above the method was not very practical as it was developed in the early work; however, the method has the potential advantage that under favourable conditions essentially all of each fatty acid component of a mixture could be obtained free from contamination, in which case three requirements of analysis could be met: the identity of each component and the quantity present in the mixture could be determined, also a considerable fraction of the pure substance itself could be obtained for further characterization. This method is especially applicable to the purification of already separated samples. Thus 0.67 % of ester could be demonstrated in a sample of mixed acids obtained by very careful saponification.<sup>11</sup> The method can probably be applied to mixtures of fatty acids using fractional elution with a graded series of eluents, a method which has been used considerably in the laboratories of Reichstein and of Ruzicka in connection with the chromatography of steroid and other mixtures. A little work of this kind has been done with fatty acids.<sup>5 16</sup> It was observed with fatty acids  $C_2$ ,  $C_3$  and  $C_4$  that the isotherms on Darco G-60 charcoal from water were well separated; however, elution analysis with water failed to yield a good separation of the three binary and one ternary mixtures investigated.<sup>16</sup> The acids were difficult to elute with water. This procedure verges on displacement analysis in certain cases.

**Frontal Analysis.**—The examination of mixtures of fatty acids by frontal analysis was reported in detail by Claesson.<sup>17</sup> He reported that by the use of a suitable adsorbent, such as Carboraffin CIV, and absolute ethanol, it was possible to obtain quantitative analyses of fatty acid mixtures containing as many as 6 fatty acids. In one experiment which was quoted a mixture of the  $n$ - $C_8$ ,  $C_9$ ,  $C_{10}$ ,  $C_{12}$ ,  $C_{14}$  and  $C_{16}$  acids was analyzed. The

<sup>12</sup> Tiselius, in *Advances in Colloid Science*, ed. E. O. Kraemer (Interscience, New York, 1942), p. 81; *Arkiv Kemi, Min. Geol. A*, 1943, **16**, No. 18.

<sup>13</sup> Martin and Synge, *Biochem. J.*, 1941, **35**, 1358.

<sup>14</sup> Zechmeister and Cholnoky, *Principles and Practice of Chromatography* (Chapman and Hall, London; John Wiley and Sons, New York, 1943).

<sup>15</sup> There is some tendency to use the word "band" for the zones on columns and paper strips and sheets. We believe that this should be discouraged if only for the practical reason that the word band is in well-established use in connection with absorption of radiation. It would seem clearer and less confusing to speak of the characteristic absorption bands observed for a component isolated from a characteristic zone on an adsorption column or paper sheet or strip than to use the word "band" in two different connections. The word seems particularly inapt in connection with the zones on paper.

<sup>16</sup> Nestler and Cassidy, *J. Amer. Chem. Soc.* (in press). This work is part of a programme supported by The Nutrition Foundation, Inc.

<sup>17</sup> Claesson, *Arkiv Kemi, Min. Geol. A*, 1946, **23**, No. 1; *Ann. N.Y. Acad. Sci.*, 1948, **49**, 183.

relative percentages of the components put into the mixture were, respectively, 10, 10, 10, 20, 20, 30; the relative per cent. found were 14, 10, 8, 22, 20, 27. Only a moderate amount of the mixture was needed, and the author states that the method is well suited for the qualitative and quantitative analysis of such fatty acid mixtures where the demands for quantitative accuracy are not too great.

Nestler and Cassidy<sup>16</sup> studied the adsorption of acetic, propionic and butyric acids upon Darco G-60 charcoal from water with a view to devising a simple analytical method for these acids, as well as to testing adsorption and chromatography theories. With frontal analysis, sharp fronts were obtained so that the number of components in the mixture could be clearly detected. However, this system did not give clear evidence that really quantitative analyses could be made with it. It was observed that the threshold, or breakthrough, volume for a given fatty acid changed with concentration of the acid, and that the two values could be related by an equation of exponential form formally resembling the Freundlich equation.

Certain practical limitations seem to be set to the acuity of frontal analyses. With any mixture the most strongly adsorbed component becomes more difficult to detect as its quantity is less or as the total concentration in the solvent is less. Increasing the amount of adsorbent is of no practical value here. The least strongly adsorbed component can be detected in relatively smaller amounts than the most strongly adsorbed. This may be because of the displacing effects of other components. Frontal analysis does have certain advantages. Under the conditions outlined by Claesson<sup>17</sup> the method requires quite small amounts of substance. For example, in one experiment with a mixture of four acids about 0.2 g. was used. Also the method is fairly rapid. With adsorbents which show some irreversible adsorption the method is still applicable where elution or displacement analysis may not be. The method is also applicable with adsorbents on which the fatty acids are adsorbed to some extent on different parts of the surface, so that they do not displace each other.

**Displacement Analysis.**—Displacement analysis of saturated fatty acids on charcoal was not found possible by Claesson.<sup>17</sup> However, Holman and Hagdahl,<sup>18</sup> using a somewhat different technique,<sup>19</sup> were able to analyze mixtures of fatty acids by displacement analysis. For example, they were able to separate lauric, myristic, palmitic and stearic acids using picric acid as the displacement agent. This work was extended<sup>20</sup> to the separation of saturated fatty acids from  $C_1$  to  $C_{20}$  and  $C_{22}$ . The separations were improved by using a different charcoal and aqueous alcohol, in which the acids are not too soluble. A higher fatty acid was utilized to displace the mixture of lower acids. Fifteen charcoals were examined as possible adsorbents, and of several of these which had good characteristics Darco G-60 was chosen. Acids  $C_1$  to  $C_5$  were separated from water, with  $C_6$  as the developer;  $C_6$  to  $C_{10}$  from 50 % ethanol, with  $C_{12}$  as developer;  $C_{10}$  to  $C_{13}$  from 65 % ethanol, with  $C_{14}$  as developer;  $C_{14}$  and  $C_{15}$  from 80 % ethanol, with  $C_{16}$  as developer;  $C_{16}$  to  $C_{20}$  from 78 % ethanol, 22 % chloroform, with  $C_{22}$  as developer. The displacement analysis of saturated, unsaturated and branched-chain fatty acids on silica gel has been reported by Claesson.<sup>21</sup>

Displacement analysis is not applicable when the adsorption isotherms of

<sup>16</sup> Holman and Hagdahl, *Arch. Biochem.*, 1948, **17**, 301.

<sup>19</sup> Hagdahl, *Acta Chem. Scand.*, 1948, **2**, 574.

<sup>20</sup> Hagdahl and Holman, *J. Amer. Chem. Soc.* (in press). Holman and Hagdahl, *J. Biol. Chem.* (submitted for publication). The authors kindly permitted us to read their manuscripts.

<sup>21</sup> Claesson, *Rec. trav. chim.*, 1946, **65**, 571.

the substances are linear.<sup>22</sup> This has been experimentally demonstrated in a clear way by Hagdahl and Holman.<sup>20</sup> They showed that the adsorption isotherms of butyric, caproic and caprylic acids are nearly linear from absolute alcohol onto Darco G-60 charcoal. With this system scarcely any separation of the acids is obtained in an attempt to displace the first two with the third. In 50 % aqueous ethanol the isotherms are well curved, and the displacement diagram shows for the different acids well-defined steps which could be recognized interferometrically as well as titrimetrically. Displacement analysis is also inapplicable where the substances are adsorbed on different parts of the surface of the adsorbent, because then one will not displace the other.

Where it is applicable, displacement analysis has a number of advantages. It allows a characterization of the components of the mixture as well as a quantitative determination of their amounts (as does frontal analysis) but in addition, like elution analysis, it yields substantial fractions of the components in pure form. This can be an important advantage since it allows confirmation of the identity of the fatty acid or other component. There is, of course, tailing under the steps of the displacement pattern,<sup>18</sup> but its effect is often of negligible importance.

**Partition Chromatography.**—This method has been applied to carboxylic acids by Smith,<sup>23</sup> Ramsey and Patterson,<sup>24</sup> Elsden,<sup>25</sup> Isherwood,<sup>26</sup> Gray,<sup>27</sup> Peterson and Johnson<sup>28</sup> and others. It would appear that this is a most promising analytical technique for fatty acids as well as dicarboxylic and substituted acids. The principle of partition chromatography may be applied using columns of adsorbent or paper strips or sheets. Ramsey and Patterson found that formic, acetic and propionic acids could be separated from each other, with butyric and isobutyric acids appearing together, when their mixtures were applied to columns of silica with water as the non-mobile phase, and chloroform-butanol as the mobile. Elsden used a silica-water-chloroform system to separate mixtures of acetic, propionic and butyric acids. Stadtman and Barker<sup>29</sup> found that quantitative separation of pentanoic, hexanoic and heptanoic acids and partial separation of heptanoic and octanoic acids could be obtained by the Elsden procedure using 0.6 % butanol in cyclohexane as the mobile solvent and Mallinkrodt's A.R. precipitated silicic acid as the support. Peterson and Johnson used Celite as the support, and water or aqueous sulphuric acid as the non-mobile phase. With benzene or chloroform-butanol mixtures as the mobile phase they were able to make separations among the fatty acids from C<sub>1</sub> to C<sub>10</sub>. In every case, of course, the mobile and non-mobile phases were mutually saturated.

Detailed descriptions have been given by Ramsey and Patterson<sup>24</sup> of techniques whereby the separation of fatty acid mixtures can be extended to acids containing as many as 19 carbon atoms. For formic, acetic, propionic and butyric acids the second solvent is butanol-chloroform, with water as the non-mobile phase. For pentanoic to decanoic acids methanol serves as the supported phase, and 2 : 2 : 4-trimethyl pentane as the second phase. For the acids from hendecanoic to nonadecanoic the supported phase is a mixture of furfuryl alcohol and 2-amino pyridine, and the mobile phase is

<sup>22</sup> Tiselius, *Arkiv Kemi, Min. Geol. A*, 1943, **16**, No. 18.

<sup>23</sup> Smith, *Biochem. J.*, 1942, **22**, P.

<sup>24</sup> Ramsey and Patterson, *J. Ass. Off. Agric. Chem.*, 1945, **28**, 644; 1948, **31**, 139, 164, 441.

<sup>25</sup> Elsden, *Biochem. J.*, 1946, **40**, 252.

<sup>26</sup> Isherwood, *ibid.*, 1946, **40**, 688.

<sup>27</sup> Gray, *J. Expt. Biol.* 1947, **24**, 10.

<sup>28</sup> Peterson and Johnson, *J. Biol. Chem.*, 1948, **174**, 775.

<sup>29</sup> Stadtman (personal communication).

hexane. Butyric and isobutyric acids which with the first set of solvents were not separated were found to be partially resolved by the methanol-iso-octane system.

**Concluding Remarks.**—What has been attempted in the limited space taken is to show examples of what has been done, to show what needs to be done, and to discuss some of the fundamental principles involved, in applying chromatography to the separation of mixtures of carboxylic acids.

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## CHROMATOGRAPHIC ANALYSIS OF FATTY OILS

BY K. A. WILLIAMS

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The application of chromatography to the analysis of fatty oils is briefly reviewed, emphasis being laid on the principles involved. The main advances of the last few years are described or referred to, particular reference being made to the separation of fatty acids from triglycerides and of hydrocarbons from the unsaponifiable matter of natural oils. The use of chromatographic analysis in the separation of the constituent molecules of natural oils is described.

The application of chromatography to the analysis of oils and fats was reviewed by the author<sup>1</sup> three years ago in a paper which traced the history of the subject and described a number of the practical applications of the method. It was emphasized that advances had been based largely on a system of trial and error, and that the principles underlying the subject were only beginning to be capable of formulation. Such formulation seemed to require a knowledge of the relative affinities of adsorbent and solvent for any given molecule, for it was rapidly becoming clear that the molecule is under the influence of competitive forces, and that their resultant determines whether or not adsorption will occur.

The resultant is moreover influenced by the presence of any other compounds which may themselves contribute to the forces acting on the adsorbable molecule, enhancing or diminishing their effect as the case may be.

The fatty oils, fatty acids, fatty alcohols and aliphatic hydrocarbons afford a remarkable set of compounds for the study of chromatography. They offer series of compounds of similar chemical structure in which the variant may be the molecular weight or the degree of unsaturation, or in which the effect of the polar carboxy group may be contrasted with that of a terminal alcoholic group, or again series in which the effect of esterification may be studied. They provide further interest by reason of the extreme difficulties they offer to chemical separation.

With hydrocarbons, the greater the degree of unsaturation, the more strongly is the compound adsorbed; conjugated double bonds lead to

<sup>1</sup> Williams, *Analyst*, 1946, **71**, 259.

greater ease of adsorption than do the same number of double bonds separated by a larger number of carbon atoms in the molecule. Thus, of the carotene isomers <sup>2</sup>  $\alpha$ -carotene, with 10 double bonds out of 11 conjugated, has least affinity for magnesium oxide;  $\beta$ -carotene, with 11 conjugated double bonds, is more strongly adsorbed; and  $\gamma$ -carotene, with 12 double bonds 11 of which are conjugated, is most strongly adsorbed. The introduction of hydroxy groups into the carotenes does not alter the order of adsorption—but it is known that it raises the general level of adsorption affinity; indeed, increase in oxygen content, in the absence of ester groupings, progressively augments it. Esterification of the xanthophylls or hydroxy-carotenes reduces their adsorption affinity almost to that of the carotenes themselves.

Of the fatty esters, whether of monohydric alcohols, such as methyl alcohol, or of polyhydric alcohols, such as glycerol, the more strongly unsaturated are the more strongly adsorbed on alumina and other adsorbents. Walker and Mills <sup>3</sup> succeeded in separating linseed oil into fractions containing 7, 6, 5 and 4 double bonds per molecule by repeated fractionation on aluminium oxide from *n*-hexane.

If hydroxy groups raise the degree of adsorption, the introduction of free carboxy groups has a pronounced effect in a different direction. The relative ease of adsorption of fatty acids of differing unsaturation is the reverse of that for the corresponding esters, both on alumina and magnesium oxide, the more saturated being more strongly adsorbed. This has been explained by the author by the postulation of polar charges of differing sign and of an adsorbent capable of attracting polar material of either positive or negative charge. Double bonds, of negative charge, and occurring in molecules otherwise non-polar, are attracted in order of increasing unsaturation; but if the molecules include a terminal group bearing a strong positive charge, such as carboxy group, and the operative affinity is the algebraic sum of the positive and negative charges, increasing unsaturation will lead to decreasing affinity if the positive element is large enough. If, on the other hand, the positive charge is not so large as the least negative charge due to unsaturation, as for instance if the terminal group is alcoholic, the algebraic sum will remain negative, and the order of adsorption will be the same as for the hydrocarbon series.

It has been noted that the reversal of the order of adsorption in a series of compounds may be brought about by changing the solvent; here again we have an example of the controlling factor being a resultant of more than one force or affinity, and there is clear evidence of an attraction between solvent and solute. Indeed, the interplay of forces of polar character becomes more generally apparent as one widens the field of observation. Chromatographic separations clearly depend on the relative strengths of attractive forces of given substances for different molecules in solution. Equally does the partition coefficient, defining the proportion in which a substance distributes itself between two solvents, depend on similar considerations. And it appears more than likely that solubility in a solvent is itself a function of the same forces.

The separation of fatty acids from triglycerides was readily and quantitatively accomplished by Sylvester, Ainsworth and Hughes <sup>4</sup> and the method has now passed into everyday use by the analyst. It is carried out on a macro scale with quantities of at least a gram of ester or acid, and depends on the fact that fatty acids are so strongly adsorbed on alumina that they cannot be eluted with ether, while fatty esters and the unsaponifiable

<sup>2</sup> Heilbron, *J. Soc. Chem. Ind.*, 1937, **56**, 160T.

<sup>3</sup> Walker and Mills, *J. Soc. Chem. Ind.*, 1942, **61**, 125; 1943, **62**, 106.

<sup>4</sup> Sylvester, Ainsworth and Hughes, *Analyst*, 1945, **70**, 295.



matter of fats remains in solution. A single passage of a solution containing triglycerides and free fatty acids in ether through an alumina column gives quantitative separation.

Monoglycerides are more strongly adsorbed by alumina than are diglycerides, and the latter in turn are more strongly adsorbed than triglycerides. Of the fatty acids themselves, the saturated acids may be separated from their mixtures, the higher members of the series being the more strongly adsorbed. This separation has been further studied by partition chromatography by Ramsey and Patterson.<sup>5</sup> Dealing with the acids with from 5 to 10 carbon atoms in the chain, they first prepared chromatographic columns of silicic acid nearly saturated with methanol containing bromocresol green and slurried with *iso*-octane. The acids were dissolved in *iso*-octane and passed through the column, when well-spaced bands were obtained, each being defined by a characteristic threshold value. It was found that in one fractionation each band was contaminated with about 5 % of the next higher homologue. The authors found that when the method was applied to a mixture of butyric and *isobutyric* acids separation was incomplete, the bands overlapping visibly. A single passage would, however, detect about 10 % of the *iso*-acid added to the normal acid or 30 % of the normal acid added to the isomer.

Partition chromatography has also been used by Ramsey and Patterson<sup>6</sup> for the separation of saturated fatty acids with from 11 to 19 carbon atoms in the chain. In this work silicic acid was used as the supporting column and was prepared by the method of Gordon, Martin and Synge.<sup>7</sup> The stationary phase consisted of a solution of 2-aminopyridine in an equal weight of purified furfuryl alcohol, 10 ml. solution being used for 20 g. silicic acid. The treated silicic acid was made to a slurry with *n*-hexane and packed into a column. The mixture of fatty acids was pipetted onto the column and allowed to sink into it under pressure. 1 ml. hexane was similarly pipetted onto the column and allowed to sink in. After a further 1 ml. had been thus added, the tube over the column was filled with hexane. After 30 ml. had percolated the liquid passing through was collected in 2-ml. portions, each of which was titrated with 0.02 N sodium ethylate. A drop of the titration to about 0.1 ml. per 2 ml. fraction was taken as evidence that a particular fatty acid had finished percolating, and the fractions representing that acid were bulked for further examination or fractionation.

Synthetic mixtures of ethyl stearate, oleate, linoleate and linolenate have been studied by Dutton and Reinbold.<sup>8</sup> Dutton had earlier separated equal parts of oleic and stearic acids on carbon, recovering 63 % oleic acid in a pure state and 53 % stearic acid. In this study the authors, however, used alumina, and the columns were developed with a solvent containing 1.75 % diethyl ether in light petroleum. Percolation was speeded by means of nitrogen pressure. Best fractionation was obtained in the stearate-linolenate system, where there is the greatest difference in unsaturation. Poorest fractionation was found in the oleate-stearate, oleate-linoleate and linoleate-linolenate systems, where there is least difference in unsaturation. In accordance with theory, the authors found that the first component eluted from a system was obtained in higher average purity than the second. The percentage recovery of the less strongly adsorbed component was usually of the order of 90, that of the more strongly adsorbed component being generally notably lower.

<sup>5</sup> Ramsey and Patterson, *J. Ass. Off. Agric. Chem.*, 1948, **31**, 164.

<sup>6</sup> Ramsey and Patterson, *J. Ass. Off. Agric. Chem.*, 1948, **31**, 44.

<sup>7</sup> Gordon, Martin and Synge, *Biochem. J.*, 1943, **37**, 79.

<sup>8</sup> Dutton and Reinbold, *J. Amer. Oil Chem. Soc.*, 1948, **25**, 120.

Reinbold and Dutton<sup>9</sup> applied a similar technique to the fractionation of soya bean oil and of ethyl esters prepared from the oil. They found a variable amount of fractionation, dependent on such factors as the quantity of oil adsorbed, the composition of the developing solvent and the activity of the adsorbent. Generally speaking, it may be said that the separations followed the course that would be predicted from knowledge of other similar work. The iodine value of successive fractions obtained from the column rose from 60.8 to a peak value of 184.6. Linolenic acid could not be detected in early fractions, but appeared in a later fraction and then continued to rise in quantity as the separation proceeded.

The use of chromatography has been extended in commercial analysis, chiefly in the field of separating hydrocarbons from triglycerides or fatty acids. This separation was first noted by Drummond<sup>10</sup> and co-worker who separated squalene from the unsaponifiable matter of olive oil by chromatographing a solution of the unsaponifiable matter in light petroleum on aluminium oxide. The method has been used by the author<sup>11</sup> for the removal of hydrocarbons from oxidation and polymerization products in the analysis of used turbine oils, and for the determination of adventitious mineral oil in wool grease.<sup>1</sup> Newberger<sup>12</sup> has worked out a method for the direct separation of mineral oil from wool grease without the previous separation of the unsaponifiable matter. It is quite clear from very many tests in my laboratory that if the unsaponifiable matter extracted from 2 to 5 g. of a fatty oil is dissolved in about 50 ml. light petroleum (b.p. 40°-60° C) and passed through an aluminium oxide column, about 1.5 cm. diam. and 20 cm. long, and the column is washed with from 150 to 200 ml. light petroleum, the percolate contains only hydrocarbons. By this means any quantity of hydrocarbon in excess of about 0.1 % may be separated from a fatty oil.<sup>13</sup>

Adventitious contamination of fatty oils has not been uncommon during the last few years. It is probably extremely rare to find any deliberate admixture, but accidental admixture is sometimes difficult to prevent. Thus, it has happened that on analysis by the method described many vegetable oils have been proved to contain mineral oil. In particular, the admixture has shown itself in linseed oil, teaseed oil, rapeseed oil and whale oil. The test may be applied to sperm oil, where traces of mineral oil are detrimental to the interests of users, and it has been shown that the method is as sensitive as for the other oils mentioned. It may be added that the residue obtained on evaporating the percolate from the chromatographic column has been found, for all the above oils when uncontaminated, to be less than 0.1 % of the weight of oil taken for the test.

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London, E.C.1.

<sup>9</sup> Reinbold and Dutton, *J. Amer. Oil Chem. Soc.*, 1948, **25**, 117.

<sup>10</sup> Thorbjarnarson and Drummond, *Analyst*, 1935, **60**, 23.

<sup>11</sup> Williams, *Analyst*, 1945, **70**, 409.

<sup>12</sup> Newberger, *J. Ass. Off. Agric. Chem.*, 1948, **31**, 670.

<sup>13</sup> Williams, *J. Ass. Off. Agric. Chem.*, 1949, **32**, 668.

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# THE APPLICATION OF PARTITION CHROMATOGRAPHY TO THE SEPARATION OF THE SUGARS AND THEIR DERIVATIVES

By E. L. HIRST AND J. K. N. JONES

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An account is given of the various applications of chromatography to the separation of mixtures of sugars and sugar derivatives. Methods of partition chromatography on strips of filter paper and on cellulose columns with automatic devices for collecting fractions are described. The uses of special solvents and developing agents (giving specific colour reactions) are discussed and methods for quantitative separation and determination of sugars on the micro scale are given. A list of  $R_f$  values for various sugars is included and examples are quoted to show the special values of these methods in the investigation of naturally occurring polysaccharides.

The similarity of the sugars in physical and chemical properties makes it difficult to separate them when mixed, and the lack of suitable methods for the separation and determination of sugars on a micro scale has delayed the progress of many investigations in plant and animal physiology. Earlier workers concentrated their efforts on the use of specific precipitants. For example, phenylhydrazine has been used to determine mannose in the form of its characteristic phenylhydrazone<sup>1</sup> and a solution of benzaldehyde in methanolic hydrogen chloride has been employed for the analysis of xylose as its dibenzylidene dimethylacetal.<sup>2</sup> Colorimetric methods have been developed for the estimation of certain sugars and their derivatives. For example, Percival and Ross<sup>3</sup> have studied the development of colour produced during the reaction of alginic acid with carbazole and sulphuric acid (the Dische reaction) and have shown that the concentration of alginic acid can be determined photometrically. Yet another line of approach has been investigated by Wise<sup>4</sup> and his colleagues, who have used certain micro organisms which ferment specifically sugars such as *D*-glucose, *D*-galactose, *L*-arabinose or *D*-xylose. The residual unfermented sugar can then be estimated by appropriate methods.

In 1939 a new approach to the problem of the separation of the sugars was made by Reich<sup>5</sup> who converted a mixture of glucose and fructose into the corresponding coloured *p*-phenylazobenzoyl derivatives and showed that their separation could be observed visually by chromatography on a column of silica or alumina. Since the description of these experiments many workers have utilized the principle of adsorption chromatography for the separation of the sugars and related substances. This method of separation was utilized by Myrbäck and Tamm,<sup>6</sup> by Freudenberg and Boppel,<sup>7</sup> by Mertzweiler, Carney and Farley<sup>8</sup> and by Coleman<sup>9</sup> to separate the *p*-phenylazo-

<sup>1</sup> Bertrand, *Compt. rend.*, 1899, **129**, 1025. Nowotowna, *Biochem. J.*, 1936, **30**, 2177.

<sup>2</sup> Breddy and Jones, *J. Chem. Soc.*, 1945, 738.

<sup>3</sup> Cameron, Ross and Percival, *J. Soc. Chem. Ind.*, 1948, **67**, 161.

<sup>4</sup> Wise and Appling, *Ind. Eng. Chem. (Anal.)*, 1944, **16**, 28. Appling, Ratcliff and Wise, *Ind. Eng. Chem. (Anal.)*, 1947, **19**, 496.

<sup>5</sup> Reich, *Biochem. J.*, 1939, **33**, 1000.

<sup>6</sup> Myrbäck and Tamm, *Svensk Kem Tidskr.*, 1941, **53**, 441.

<sup>7</sup> Freudenberg and Boppel, *Ber.*, 1940, **73**, 609.

<sup>8</sup> Mertzweiler, Carney and Farley, *J. Amer. Chem. Soc.*, 1943, **65**, 2367.

<sup>9</sup> Coleman, *J. Amer. Chem. Soc.*, 1942, **64**, 1501.

benzoyl derivatives of the fully and partially methylated sugars. Alumina was used as the adsorbent in these experiments, and the work was extended by Coleman, Farnham and Miller,<sup>10</sup> Coleman *et al.*<sup>11</sup> and Hurd and Zelinsky,<sup>12</sup> who employed similar methods for the separation of simple mixtures of the mono- and di-saccharides. Boissonas<sup>13</sup> has described yet another method for the separation of small quantities of the methylated sugars. They were first hydrogenated to the corresponding hexitol derivatives, and then separated on a column of alumina after conversion to the *p*-phenylazobenzoates, difficulties due to the presence of the different ring forms of the sugars being thus avoided.

McNeely, Binkley and Wolfrom<sup>14</sup> have used Magnesol, a hydrated magnesium silicate, as an adsorbent for the separation of mixtures of the sugar acetates. Since these substances are colourless it is necessary to extrude the column of Magnesol and locate the position of the sugar derivatives by streaking the side of the column with permanganate solution (the Zechmeister brush technique). Lew, Wolfrom and Goepf<sup>15</sup> applied this procedure to the chromatography of unsubstituted sugars and related compounds on a type of fuller's earth, and the method has been utilized to separate the components of cane juice, black strap molasses, the degradation products of streptomycin and many other mixtures of sugars. Using hydrated calcium acid silicate and a similar technique, Georges, Bower and Wolfrom<sup>16</sup> were able to separate mixtures of sugar acetates and of methylated sugars.

The chromatographic separation of starch into its components, amylose and amylopectin, has been achieved by Pacsu and Müller<sup>17</sup> and by Ashford, Evans and Hibbert<sup>18</sup> who have confirmed the earlier results of Tanret.<sup>19</sup> Levi<sup>20</sup> has shown that polysaccharides can be separated on adsorbents such as magnesium oxide and calcium carbonate. Glycogen is easily separated, since it is not eluted by hot water and is located on the column by its white fluorescence. Inulin, on the other hand, shows a blue fluorescence. Mark and Saito<sup>21</sup> have used chromatography to fractionate the acetates of cellulose making use of the observation that materials of lower molecular weight were adsorbed more tenaciously than those of high molecular weight. Dickey and Wolfrom<sup>22</sup> separated the products of acetolysis of cellulose by chromatography on Silene E.F. and Magnesol, derivatives of glucose, cellobiose and cellotriose being isolated.<sup>23</sup>

Tiselius,<sup>24</sup> and Tiselius and Hahn<sup>25</sup> have utilized the principle of the flowing chromatogram to separate complex mixtures of sugars. A 0.5 % aqueous solution of ephedrine was used to develop the sugars adsorbed on a column of activated carbon. The elution and separation of the sugars was followed by observing the change in refractive index of the eluate. The temperature of the entire apparatus was thermostatically controlled to

<sup>10</sup> Coleman, Farnham and Miller, *J. Amer. Chem. Soc.*, 1943, **65**, 1588.

<sup>11</sup> Coleman, Rees, Sundberg and McCloskey, *J. Amer. Chem. Soc.*, 1945, **67**, 381.

<sup>12</sup> Hurd and Zelinsky, *J. Amer. Chem. Soc.*, 1947, **69**, 243.

<sup>13</sup> Boissonas, *Experientia*, 1947, **3**, 1; *Helv. chim. Acta*, 1947, **30**, 1689, 1703.

<sup>14</sup> McNeely, Binkley and Wolfrom, *J. Amer. Chem. Soc.*, 1945, **67**, 527.

<sup>15</sup> Lew, Wolfrom and Goepf, *J. Amer. Chem. Soc.*, 1945, **67**, 1865.

<sup>16</sup> Georges, Bower and Wolfrom, *J. Amer. Chem. Soc.*, 1946, **68**, 2169.

<sup>17</sup> Pacsu and Müller, *J. Amer. Chem. Soc.*, 1941, **63**, 1168.

<sup>18</sup> Ashford, Evans and Hibbert, *Can. J. Res. B*, 1946, **24**, 246.

<sup>19</sup> Tanret, *Compt. rend.*, 1914, **158**, 1353.

<sup>20</sup> Levi, *Rev. brasil. Chem.*, 1940, **10**, 113.

<sup>21</sup> Mark and Saito, *Monatsh.*, 1936, **68**, 237.

<sup>22</sup> Dickey and Wolfrom, *J. Amer. Chem. Soc.*, 1949, **71**, 825.

<sup>23</sup> Montgomery, Weakley and Hilbert, *J. Amer. Chem. Soc.*, 1949, **71**, 1682.

<sup>24</sup> Tiselius, *Advances in Colloid Science*, 1942, **1**, 81; *Kolloid-Z.*, 1943, **105**, 101.

<sup>25</sup> Tiselius and Hahn, *Kolloid-Z.*, 1943, **105**, 177.

avoid intermixing of the layers of separated sugar solutions. A similar procedure was used by Claesson,<sup>26</sup> who showed that synthetic polymers, and derivatives of cellulose such as the nitrate, can be fractionated by chromatography. In the course of this work it was found that with carbon as adsorbent substances of high and low molecular weight were adsorbed less tenaciously than those fractions of intermediate molecular weight.

Trimethyl and tetramethyl methylglucosides have been separated on a column of alumina using a flowing chromatogram.<sup>27</sup> The glycosides were developed with a mixture of ether and light petroleum and the eluate tested for the presence of carbohydrate by the Molisch reagent. A partial separation of the trimethyl derivatives of methyl xyloside and methyl arabinoside was also obtained. Norberg, Auerbach and Hixon<sup>28</sup> observed that 2:3-dimethyl-, 2:3:6-trimethyl- and 2:3:4:6-tetramethyl-glucose could be separated one from another by adsorption chromatography on activated fibrous alumina, using acetone-benzene mixture as developer. The position of the sugars on the column was detected by their fluorescence in ultra-violet light.

With the introduction of partition chromatography (1941) a further method for the separation of the sugars became available. Bell,<sup>29</sup> who was the first (1944) to apply this new procedure, showed that 2:3:4:6-tetramethyl glucose and 2:3:6-trimethyl glucose could be separated from one another and from 2:3-dimethyl glucose by partition chromatography on a column of silica gel with water as the stationary phase, using first chloroform and then chloroform-*n*-butanol as the mobile phase. The tetramethyl glucose travelled rapidly through the column when chloroform was used as eluant, the trimethyl and dimethyl glucose remaining at the top; on changing the solvent to chloroform-*n*-butanol, the trimethyl glucose moved through the column and appeared in the eluate. The position of the dimethyl glucose which remained on the column was then determined by extrusion and streaking with Molisch reagent.

A further advance came with the application of paper partition chromatography to the analysis of complex mixtures of simple sugars, since this method enables detailed studies of the composition of complex polysaccharides to be performed using only minute amounts of material. Originally, the method of paper partition chromatography was developed by Consden, Gordon and Martin<sup>30</sup> for the analysis of protein and peptide hydrolyzates, but in 1946 it was applied by Partridge<sup>31</sup> to the separation of the sugars. It was shown that very small quantities (*ca* 10  $\mu$ g.) of sugar mixtures could be separated, and their components identified, by virtue of the difference in partition of the sugars between water held by the cellulose fibres of the paper (the stationary phase) and a solvent such as butanol saturated with water (the mobile phase). The procedure consists in placing a spot of a sugar solution near the top of a sheet or strip of filter paper, the top of which is immersed in a suitable solvent mixture contained in a trough. The trough and paper are then placed in a container so that the paper hangs vertically from the trough, and the atmosphere which surrounds it is saturated with the vapour of the solvents and with water vapour. The solvent is then allowed to advance down the paper until the horizontal front reaches the bottom, when the paper is removed and dried. The sugars are identified by the rate

<sup>26</sup> Claesson, *Chem. Soc. Discussion on Chromatography*, 1948; *Ann. N.Y. Acad. Sci.*, 1948, **49**, 183.

<sup>27</sup> Jones, *J. Chem. Soc.*, 1944, 333.

<sup>28</sup> Norberg, Auerbach and Hixon, *J. Amer. Chem. Soc.*, 1945, **67**, 342.

<sup>29</sup> Bell, *J. Chem. Soc.*, 1944, 473.

<sup>30</sup> Consden, Gordon and Martin, *Biochem. J.*, 1944, **38**, 224.

<sup>31</sup> Partridge, *Nature*, 1949, **158**, 270; *Biochem. J.*, 1948, **42**, 238.

TABLE I

SOLVENT-*n*-BUTANOL 50 PARTS, ETHANOL 10 PARTS, WATER 40 PARTS (TOP LAYER)

Substance	$R_f$ value	Substance	$R_f$ value
Raffinose .. .. .	0.001	2 : 4-dimethyl galactose ..	0.41
Lactose .. .. .	0.016	4 : 6-dimethyl galactose ..	0.42
Maltose .. .. .	0.021	2-deoxy ribose, 2 : 6-dimethyl- galactose .. .. .	0.44
Sucrose .. .. .	0.03	4 : 6-dimethyl glucose ..	0.46
<i>D</i> -gluco- <i>L</i> -gala-octose ..	0.038	2-methyl fucose ..	0.51
<i>D</i> -gulo- <i>L</i> -gala-heptose ..	0.050	3 : 6-dimethyl glucose }	0.52
<i>D</i> -gluco- <i>D</i> -gulo-heptose ..	0.053	3 : 4-dimethyl glucose }	0.54
<i>D</i> -manno- <i>D</i> -gala-heptose ..	0.058	4 : 6-dimethyl altrose }	0.57
<i>D</i> -gala- <i>L</i> -gluco-heptose ..	0.064	2 : 3-dimethyl mannose ..	0.58
Turanose .. .. .	0.060	2 : 3-dimethyl glucose }	0.60
Galactose .. .. .	0.070	4-methyl rhamnose ..	0.61
<i>D</i> -gluco- <i>L</i> -talo-octose ..	0.082	4 : 6-dimethyl mannose }	0.64
Glucose .. .. .	0.090	3 : 4-dimethyl mannose ..	0.66
Sorbosc .. .. .	0.10	3-methyl quinovose ..	0.67
Mannose, manno-heptulose }	0.11	3 : 4-dimethyl fructose }	0.68
<i>D</i> -gala- <i>L</i> -manno-heptose }	0.12	2-deoxy rhamnose ..	0.71
<i>D</i> -gulo- <i>L</i> -talo-heptose }	0.15	2 : 3-dimethyl arabinose }	0.74
Fructose, Gluco-heptulose }	0.16	2 : 3 : 4-trimethyl galactose }	0.76
Gulose, Arabinose, Tagatose }	0.17	2 : 4-dimethyl xylose ..	0.79
Xylose .. .. .	0.18	2 : 4 : 6-trimethyl galactose ..	0.81
4-methyl galactose ..	0.19	3-methyl altromethylose ..	0.83
Altrose .. .. .	0.21	2 : 3 : 6-trimethyl galactose ..	0.84
Idose, 6-methyl galactose ..	0.22	2 : 3-dimethyl xylose ..	0.85
Talose, Lyxose .. ..	0.23	2 : 4 : 6-trimethyl glucose ..	0.86
Ribose, Fucose .. ..	0.25	3 : 4 : 6-trimethyl mannose ..	0.87
2-methyl glucose .. ..	0.26	2 : 3 : 6-trimethyl glucose }	0.88
2-methyl galactose .. ..	0.27	1 : 3 : 4-trimethyl fructose }	0.90
Riboketose, Apiose, 2-deoxy- galactose .. .. .	0.28	3 : 4-dimethyl rhamnose ..	0.94
3-methyl glucose .. ..	0.30	2 : 3 : 4-trimethyl glucose ..	0.95
Xyloketose .. .. .	0.32	3 : 4 : 6-trimethyl fructose ..	0.96
6-methyl glucose .. ..	0.33	Cymarose .. .. .	1.00
Quinovose .. .. .	0.34	Oleandrose, 2 : 3 : 4 : 6-tetra- methyl galactose ..	0.96
Rhamnose .. .. .	0.37	Tetramethyl fructopyranose ..	0.99
$\alpha$ -methyl mannoside ..	0.38	2 : 3 : 4-trimethyl xylose ..	1.01
3 : 4-dimethyl galactose }		2 : 3 : 5-trimethyl arabinose ..	
4-methyl mannose ..		2 : 3 : 4 : 6-tetramethyl man- nose .. .. .	
$\beta$ -methyl arabinoside }		2 : 3 : 4 : 6-tetramethyl glucose }	
2-deoxy allose .. ..		2 : 3 : 5 : 6-tetramethyl glu- cose .. .. .	
2-methyl $\beta$ -methyl altroside ..		2 : 3 : 4-trimethyl rhamnose }	
Rhamnoketose, 3 : 6-anhydro- glucose, talo methylose ..		1 : 3 : 4 : 6-tetramethyl fruc- tose .. .. .	
2-methyl xylose, 2-methyl ara- binose .. .. .			

The rates of movement ( $R_f$  values) of the methylated sugars are determined by dividing the distance the sugars have moved from the starting line by the distance moved by tetramethyl glucopyranose ( $R_f = 1$ ) under the same conditions. For the unsubstituted sugars it is convenient to use rhamnose as standard. A sugar cannot always be identified with certainty by this procedure alone, since a number of the methylated sugars travel to the same or similar position on the paper chromatogram and the  $R_f$  values are not exactly reproducible, but alter slightly with variations in apparatus and conditions. We have found, for example, that these values vary slightly from one apparatus (enclosed in a bell jar) to another (enclosed in a tank). Identification can be facilitated, however, by using several standards and calculating the  $R_f$  value of the unknown sugar from that of the nearest known sugar on the chromatogram. Wherever possible, the rate of movement of the unknown sugar should be compared on the same chromatogram, with that of a known specimen.

at which they move under standard conditions down the face of the paper (see Table I) (Partridge, *loc. cit.*, Brown, Hirst, Hough, Jones and Wadman,<sup>32</sup> Hirst, Hough and Jones,<sup>33</sup> Jermyn and Isherwood<sup>34</sup>). The positions of the reducing sugars are readily detected by the reduction of ammoniacal silver nitrate (Partridge) or by the colours developed with aniline hydrogen phthalate<sup>34a</sup> or *o*-dinitrobenzene and alkali. They may also be detected and in some cases identified by the colours produced on warming with reagents<sup>35</sup> such as trichloroacetic acid and aniline,  $\alpha$ -naphthylamine, diphenylamine, dimethylaniline, *o*-anisidine, *o*-toluidine,  $\alpha$ -naphthylamine 6:8-disulphonic acid or urea. For example, diphenylamine serves to differentiate between trimethyl xylopyranose, which gives an intense mauve colour, and trimethyl arabinofuranose, which gives a pale green. Urea gives a black colour only with ketoses. Observation of the colours and examination of their fluorescence under ultra-violet light helps to differentiate between various sugars and their derivatives and this procedure is of particular value in the examination of mixtures of methylated sugars (Hough, Jones and Wadman<sup>36</sup>). Forsyth<sup>37</sup> has used a naphtharesorcinol and hydrochloric acid spray; *m*-phenylene diamine<sup>37a</sup> and benzidine<sup>37b</sup> have also been used. The identification of the sugars is, in some cases, facilitated by an examination of the pattern of sugars produced after epimerization of the unknown sugar with lime water and examination of the products on the paper chromatogram (Hough, Jones, and Wadman<sup>38</sup>).

The separation of sugars by partition chromatography was later used as the basis of a method for the quantitative separation and estimation of the sugars.<sup>34 39</sup> Providing the separation of the sugars is complete, an oxidizing agent that will deal with micro quantities serves for the estimation of each of the components. In order to obtain measurable quantities of sugar, a number of spots of the sugar solution are placed on a line drawn parallel to and about 4 in. from the edge of a sheet of Whatman No. 1 filter paper. The sugars are then separated in the manner described above. The positions of the separated sugars are then determined by cutting narrow strips from the sides and centre of the dried paper, spraying them with a suitable reagent and heating. By reference to the sprayed strips, it is possible to cut the paper into areas, each of which contains one of the separated sugar components. The sugars (*ca.* 0.1–1.0 mg.) are extracted with water in a micro Soxhlet apparatus and are then estimated with either Somogyi's micro copper reagent,<sup>40</sup> with sodium hypoiodite, or with sodium periodate solution.<sup>41</sup> This method has recently been extended to the separation and determination of mixtures of the methylated sugars (Hirst, Hough and Jones<sup>33</sup>) (see Table II). Hawthorne<sup>39</sup> has used a similar method to separate and determine even smaller quantities (40–100  $\mu$ g.) of sugars. In this instance the sugar solutions (4.0  $\mu$ l.) were placed on the paper by means of an accurately calibrated micropipette. After separation the sugars were collected in caps

<sup>32</sup> Brown, Hirst, Hough, Jones and Wadman, *Nature*, 1948, **161**, 720.

<sup>33</sup> Hirst, Hough and Jones, *J. Chem. Soc.*, 1949, 928.

<sup>34</sup> Jermyn and Isherwood, *Biochem. J.*, 1949, **44**, 402.

<sup>34a</sup> Partridge, *Nature*, 1949, **164**, 443.

<sup>35</sup> Hough, Jones and Wadman (in press).

<sup>36</sup> Hough, Jones and Wadman (in press).

<sup>37</sup> Forsyth, *Nature*, 1948, **161**, 239.

<sup>37a</sup> Chargaff, Levine and Green, *J. Biol. Chem.*, 1948, **175**, 67.

<sup>37b</sup> Horrocks, *Nature*, 1949, **164**, 444.

<sup>38</sup> Hough, Jones and Wadman (unpublished results).

<sup>39</sup> Flood, Hirst and Jones, *Nature*, 1947, **160**, 865; *J. Chem. Soc.*, 1948, 1679. Hawthorne, *Nature*, 1947, **160**, 714.

<sup>40</sup> Somogyi, *J. Biol. Chem.*, 1945, **160**, 61.

<sup>41</sup> Hirst and Jones, *J. Chem. Soc.*, 1949, 1659.

cut in paraffin wax and determined by the method of Linderstrøm-Lang and Holter.<sup>42</sup> In another procedure,<sup>43</sup> the area of the spots produced on spraying the sugars on the paper with Tollens reagent was determined and, by comparison with standards, the amount of sugar estimated.

Separation of the sugars on a micro scale by partition chromatography often facilitates their identification, but the method will not differentiate between the *D* and *L* stereo-isomers of the same sugar. To overcome this difficulty larger quantities of material are necessary and Hough, Jones and Wadman<sup>44</sup> devised an automatic apparatus with the aid of which mixtures of simple sugars can be separated on a semi-micro scale on a column of cellulose using solvents such as *n*-butanol-water mixture, isopropyl alcohol-water, acetone-water or ethanol. In this procedure, the mixture of sugars to be analyzed is placed on top of a column of cellulose, which has been treated

TABLE II

% OF SUGARS (CALCULATED AS HEXOSAN, METHYL PENTOSAN AND PENTOSAN RESPECTIVELY) PRODUCED AFTER HYDROLYSIS WITH BOILING N SULPHURIC ACID FOR 5 HR.

Polysaccharide	Galactose	Arabinose	Xylose	Rhamnose
Larch $\epsilon$ -Galactan <sup>45</sup> .. .. .	82.1	10.6	—	—
Damson Gum <sup>46</sup> .. .. .	30.8	37.9	4.9	3.3(?)
Cherry Gum ( <i>Prunus avium</i> , <sup>47</sup> from Italy) .. .. .	21.8	48.0	5.5	+
Cherry Gum (from a double white ornamental variety) .. .. .	24.0	48.8	3.6	+
Peach Gum (from Italy) ( <i>Prunus persica</i> ) .. .. .	37.8	38.6	13.8	ca. 5

## Glucose derivatives

Polysaccharide	2 : 3 : 4 : 6-tetramethyl	2 : 3 : 6-trimethyl	2 : 3-di-methyl	? : 6-di-methyl	mono-methyl
Methylated Waxy Maize Starch .. .. .	4.2	80.0	4.0	10.0	1.3
Methylated rabbit liver Glycogen .. .. .	8.7	69.0	8.9	10.8	2.4

For details of the procedure used in the analysis of polysaccharides and their methylated derivatives, see ref. <sup>43</sup>.

previously with the developing solvent. The solvent is allowed to percolate down the column and the eluate is collected in an apparatus which changes the receiver automatically at predetermined intervals. The apparatus is designed so that the time intervals may be varied at will and so that four columns can be operated per turntable. In the present design of apparatus, the control unit will operate three tables simultaneously at different speeds, so that no less than twelve analyses can be carried out at one and the same time. Some flexibility is necessary as the rate of flow of solvent through the columns of cellulose varies with the size and density of packing of the column and with the solvent used.

<sup>42</sup> Linderstrøm-Lang and Holter, *C.R. Lab. Carlsberg*, 1933, **19**, No. 14.

<sup>43</sup> Fisher, Parsons and Morrison, *Nature*, 1948, **161**, 764.

<sup>44</sup> Hough, Jones and Wadman, *Nature*, 1948, **162**, 448.

<sup>45</sup> Campbell, Hirst and Jones, *J. Chem. Soc.*, 1948, 774.

<sup>46</sup> Hirst and Jones, *J. Chem. Soc.*, 1938, 1174.

<sup>47</sup> Jones, *J. Chem. Soc.*, 1939, 558.



In this manner, a large number of fractions of eluate are collected. Spots of the eluate from each fraction are then placed in chronological order on the starting line of a sheet filter paper chromatogram, and the sugars are separated on the paper in the usual way. After drying, their positions are shown by spraying the paper with a suitable reagent, and then heating it. The degree of separation is thereby ascertained and the tubes which contain pure sugars can be selected. Since the composition of mixed intermediate fractions can be determined, a complete analysis of the mixture can be effected. In a similar way, mixtures of the methylated derivatives of sugars may be separated and identified.<sup>48</sup> For such mixtures the solvent *n*-butanol-water is useful for the separation of sugars containing less than 40 % of methoxyl groups and *n*-butanol-light petroleum (b.p. 100°-120°) is preferable for the separation of sugars having a value of OMe greater than 40 %. In some instances, separation of the hydrolysis products of a methylated gum has led to the isolation of small fractions of unknown constitution. The identification of such fractions has been facilitated by demethylation<sup>49</sup> with hydrobromic acid and subsequent analysis of the sugars produced on a sheet paper chromatogram. Tetramethyl glucose is readily converted into glucose, trimethyl xylose into xylose and 2 : 6-dimethyl galactose is converted into galactose by this procedure. Fructose derivatives, however, are destroyed.

Separation on a cellulose column has led to the isolation of *D*-tagatose, *D*-galactose and *L*-rhamnose<sup>50</sup> from the mixture of sugars produced on hydrolysis of *Sterculia setigera* gum, and to the isolation of pure specimens of *D*-quinovose, *D*-fucose, *D*-glucose and an unknown sugar from the hydrolysis products of convulvulinic acid.<sup>51</sup> Formose,<sup>52</sup> the mixture of sugars produced on heating formalin with calcium carbonate, has been shown<sup>53</sup> to contain at least six sugars, some of which are ketopentoses in addition to material of higher and lower molecular weight.

The uronic acids and their derivatives may also be separated on a column of cellulose, a mixture of *n*-butanol, glacial acetic acid, *n*-butyl acetate and water being employed as the mobile phase. The aniline-trichloroacetic acid reagent gives a deep red colour with the methylated derivatives of uronic acids.

The examples given are sufficient to indicate that problems involving the separation and determination of sugars have been very greatly facilitated by the use of partition chromatography and these new techniques which are applicable on the micro scale provide a powerful and much-needed weapon for an attack on many biochemical problems.

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<sup>48</sup> Hough, Jones and Wadman, *J. Chem. Soc.*, 1949, 2511.

<sup>49</sup> Hess and Neumann, *Ber.*, 1935, **68**, 1371. Araki and Hasi, *J. Chem. Soc., Japan*, 1939, **60**, 774 (cf. *A.C.S. Abstr.*, 1942, **36**, 4483, 5146).

<sup>50</sup> Hirst, Hough and Jones, *Nature*, 1949, **163**, 177.

<sup>51</sup> Jones and Stacey (unpublished).

<sup>52</sup> von Euler, *Ber.*, 1906, **39**, 39, 48.

<sup>53</sup> Byers and Jones (unpublished).

# THE CHROMATOGRAPHY OF PROTEINS. THE EFFECT OF SALT CONCENTRATION AND pH ON THE ADSORPTION OF PROTEINS TO SILICA GEL

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The adsorption of an ovalbumin and a serum albumin preparation on silica gel was dependent upon the salt concentration, and increasing ionic strength in the range of 0.025 to 3 resulted in increased adsorption. The adsorption of two globulin preparations was not so dependent on salt concentration, and the immune globulin showed maximal adsorption at all salt concentrations studied. The adsorption of all the preparations appeared to reach approximately the same maximal values.

The adsorption of the ovalbumin and the immune globulin was also dependent upon the pH, the ovalbumin demonstrating maximal adsorption at pH 4.0 and 5.0, decreasing adsorption at pH 6.0, and little if any at pH 7.0, 7.5, and 8.3, while the immune globulin exhibited maximal adsorption from pH 4.0 to 7.5 and decreased adsorption only at pH 8.3. These results were obtained at an ionic strength of 0.1.

Chromatography of proteins in columns of silica gel and Supercel showed that the serum albumin and immune globulin could be distinctly separated in a solvent of sodium phosphate at pH 7.0 and an ionic strength of 0.1. In chromatograms of human serum under these conditions albumin and globulin were distinctly separated.

Several other substances were studied for their ability to adsorb protein. Although "salting-out" adsorption in paper strips chromatograms was demonstrable, the adsorption by paper in batch experiments was not detectable. Although potato starch manifested adsorption of acid dyes, no adsorption of proteins could be shown. No adsorption of proteins was seen on aluminium hydroxide, but weak adsorption was observed on Supercel and with carbon adsorption was stronger than with silica gel.

The investigation of proteins has depended much upon the analytical procedure available. Chromatographic analysis has provided much help in the study of other substances, for example, the amino acids, yet the application of chromatography to protein has not been extensive. That proteins are adsorbed from aqueous solutions onto various solids is well known, and the phenomenon is frequently made use of in batch operations in purification procedures. The solution is shaken with a certain amount of finely divided adsorbent and the adsorbent removed by filtration or sedimentation. Usually conditions are arranged so that it is contaminating protein which is adsorbed and the substance to be purified remains in solution, since elution of adsorbed protein is frequently difficult and may require the use of solvents which are injurious to the protein. However, such batch adsorptions do not lend themselves easily to analytical work and the advantages of chromatographic analysis are not made use of.

Another approach to the problem was indicated by Tiselius who described "salting-out" adsorption and showed that the presence of salts, such as ammonium sulphate and potassium phosphate, promoted the adsorption of certain proteins and acid dyes onto silica gel and paper.<sup>1</sup> The importance

<sup>1</sup> Tiselius, *Arkiv Kemi, Min. Geol. B*, 1948, **26**, No. 1.

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of salt concentration was shown by examples where adsorption was marked in the presence of salts even though little or no adsorption could be demonstrated in their absence. In the present paper this work has been extended and some of the factors affecting adsorption have been investigated. Some chromatographic analyses are reported.

### Experimental

In general, two experimental approaches were employed. In the first, batch experiments were done in which solutions were mixed with an adsorbent until adsorption appeared complete, the adsorbent removed by centrifugation or filtration and the protein concentration determined in the remaining solution. The concentration of protein was measured by determining its optical density in a Beckman spectrophotometer at 278  $m\mu$ . This method of measurement imposed certain precautions because the optical density values obtained at this wavelength are increased by the presence of finely divided particulate matter. Since it was desirable to have the adsorbent in a finely divided state in order to promote adsorption, it was necessary to employ procedures which removed the adsorbent efficiently. Another source of suspended material was found in experiments where shaking was done in too vigorously a fashion and caused some denaturation to take place, so that a finely divided suspension of insoluble protein resulted. This last source of difficulty could be avoided by carrying out adsorption in flasks which were rotated so that the fluid travelled around the bottom of the flask and kept the adsorbent well suspended but yet no foaming or bubble formation took place. Spectrophotometric measurements were done at 320, 278 and 260  $m\mu$  with water in the control cell. Non-specific optical adsorption revealed itself by increased values at 320 and 260  $m\mu$ . A ratio between the optical densities at 260 and 278  $m\mu$  of less than 0.7 was observed with all clear protein preparations before adsorption. With adsorbed preparations such a ratio indicated satisfactory conditions and was always obtained except at times when very little protein remained in solution.

Shaking was carried out for 30 min. since it was observed that absorption was nearly complete at this time. In one experiment with the serum albumin at 0.1 %, pH 7.7 and  $\mu$  (ionic strength) = 0.125, the amount of protein absorbed after 5, 15, 30, 60 and 120 min. was 27, 30, 32, 33 to 35 %, respectively.

The second general approach employed, and the one toward which the batch experiments were directed, was chromatographic analysis in columns. Appropriate solutions were passed through a column of adsorbent contained in filters which were essentially the same as those described by Claesson<sup>2</sup> but were made of Perspex instead of metal. The filters have dimensions which give them satisfactory height/diameter ratios and their volumes are arranged to be multiples of  $\pi$ . Thus a filter whose volume is said to be  $250\pi$  has a volume of  $250\pi$  mm.<sup>3</sup>. The effluent was collected in a fraction collector designed by Claesson (unpublished). With this fraction collector the effluent drains into a container which, when filled, siphons into a test-tube, where the weight of the fluid actuates a wheel which carries a new tube into position below the siphon. With the siphon used the volume drained was about  $3\frac{1}{2}$  ml., but the variation between successive drainings was several tenths of a millilitre. This variation affects the results presented in the charted chromatographic analyses by giving some uncertainty to the abscissa value, which is not important in the work here presented. For details on the theory and practice of chromatography as it is carried out here the reader is referred to two reviews.<sup>2, 3</sup>

Most of the protein preparations used were obtained through the courtesy of Dr. K. O. Pedersen. The immune serum globulin was from humans and was an American commercial product which had been prepared by alcohol fractionation. The serum globulin was a bovine product obtained by precipitation at neutral reaction at ammonium sulphate concentration between 35 % and 45 % saturation. The serum albumin was also a bovine product and was obtained by precipitation with neutral ammonium sulphate at greater than 60 % saturation.

<sup>2</sup> Claesson, *Arkiv Kemi, Min. Geol. A*, 1946, **23**, No. 1.

<sup>3</sup> Tiselius, *Advances in Protein Chemistry*, 1947, **3**, 67.

The ovalbumin was a thrice-crystallized product. All experiments were carried out at room temperature, which varied from 19°–22° C.

### Results

**Silica Gel as Adsorbent.**—Before efforts were concentrated on silica gel, some attempts were made to chromatograph serum proteins in paper strips. Adsorption could be demonstrated when salt concentrations close to salting-out concentration were employed with protein concentration of about 0.1 %. However, it was difficult to work with lower concentration of protein because, after the spreading which always occurred, the boundaries were not clearly visible with the tests used to detect protein, which were the nitrosonaphthol test for tyrosine and bromphenol blue test,<sup>4</sup> both modified for use on paper. Several adsorbents were then compared and it was observed that although salting-out adsorption of acid dyes occurred on starch, salting-out adsorption of protein was weak on this adsorbent. However, adsorption of proteins proved to be strong on silica gel.

**Preparation of Silica Gel.**—A number of samples of silica gel from different manufacturers were investigated, using the coloured protein phycoerythrin. It was found that there was considerable variation between samples but that the chief reason for the variation lay in their alkalinity or acidity. The samples which were strongly alkaline adsorbed phycoerythrin poorly even in the presence of 0.5 M ammonium sulphate, while those which were strongly acid adsorbed strongly even in the absence of ammonium sulphate. Other samples of intermediate reaction adsorbed phycoerythrin only in the presence of ammonium sulphate. Accordingly an available silica gel (acid silicic, powd., Bakers' Analyzed) which appeared satisfactory was selected and used for the rest of the experiments. It was also found that additional grinding increased the speed of absorption. The following method of preparation was adopted and followed for the work hereafter described. The silica gel was ground wet in a ball mill for 8 to 16 hr., suspended in five to ten times its volume of water and allowed to settle overnight. It was then re-suspended in water and the reaction of the suspension adjusted to approximate neutrality with sodium hydroxide. The silica gel was washed until the supernatant was nearly clear by allowing it to settle 12 to 24 hr. and decanting the supernatant. The largest particles were eliminated by discarding the sediment which settled in about  $\frac{1}{2}$  hr. From such stock suspensions lots for particular experiments were prepared by suspending an amount of silica gel in the buffer which was to be used, adjusting to the pH desired by the addition of sodium hydroxide or hydrochloric acid, and washing the silica gel three or four times by centrifugal sedimentation and re-suspension in fresh buffer, the final sediment being dried by pressing between filter papers and drying at 105° overnight. The resulting dry powder appeared to be stable in that no change in adsorptive properties was observed during the course of several months.

**Effect of Salt Concentration on Adsorption of Proteins.**—The effect of salt concentration was investigated with the results which are summarized in Fig. 1. These results were obtained in batch experiments in which 5 ml. solution were mixed for 30 min. with 100 mg. silica gel washed in the buffer and dried as described above. The same solutions to which no silica gel was added served as control, and the amount of adsorption was determined by subtracting the optical density at 278 m $\mu$  of the adsorbed solution from the control solution. In order to regulate the pH, all solutions contained sodium phosphate buffer with a ratio of 27/19 of disodium to monosodium phosphate. At  $\mu = 0.1$  this buffer gives a pH of 6.99. The sodium chloride and sodium sulphate solutions were neutral before the addition of sodium phosphate.

All solutions contained enough phosphate buffer to give an ionic strength of 0.1 except the three shown at the far left in Fig. 1, whose ionic strengths were 0.025. The solutions with ionic strengths greater than 0.1 were obtained by adding sodium chloride or sodium sulphate to the sodium phosphate so that the total ionic strengths were those indicated.

<sup>4</sup> Feigl, *Qualitative Analysis by Spot Tests* (New York, 1947).

The eight salt solutions used had the following salt compositions: (1) 0.001 M monosodium phosphate + 0.008 M disodium phosphate, (2) 0.004 M monosodium phosphate + 0.032 M disodium phosphate, (3) 0.25 M sodium chloride + 0.004 M, monosodium phosphate + 0.032 M disodium phosphate, (4) 0.10 M sodium sulphate + 0.004 M monosodium phosphate + 0.032 M disodium phosphate, (5) 0.25 M sodium sulphate + 0.004 M monosodium phosphate + 0.032 M disodium phosphate, (6) 0.50 M sodium sulphate + 0.004 M monosodium phosphate + 0.032 M disodium phosphate, (7) 0.75 M sodium sulphate + 0.004 M monosodium phosphate + 0.032 M disodium phosphate and (8) 1.0 M sodium sulphate + 0.004 M monosodium phosphate + 0.032 M disodium phosphate.

The silica gel was removed from the adsorption mixtures by two centrifugations. The controls were also centrifuged. It will be seen by examination of Fig. 1 that the effect of the salt concentration depends on the protein employed. In the case of ovalbumin there is increasing adsorption in the whole range. On the other hand with the immune globulin little change is seen with variation in ionic strength. The serum globulin falls between these two as does the serum albumin.

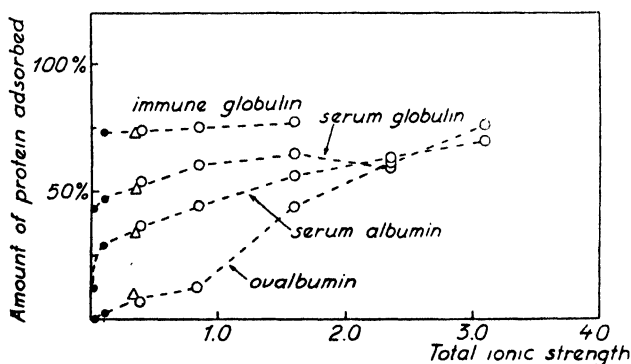


FIG. 1.—The effect of salt concentration on the amount of protein adsorbed onto silica gel from a 0.1% solution of the protein indicated. The salts used were sodium phosphate (●), sodium chloride + sodium phosphate (Δ) and sodium sulphate + sodium phosphate (○). All solutions contained sodium phosphate at an ionic strength of 0.1, except the three at the far left, where the ionic strength was 0.025. pH 7.0 with  $\mu = 0.1$ .

The serum albumin used behaved in this and other experiments as if it contained two components widely separated in adsorbability. It will be noted that some of the values are not reported for the two globulins. This arises from the fact that at certain salt concentration these proteins were not completely soluble and the control tubes, those containing no silica gel, showed a precipitate before centrifugation.

The most instructive results were those obtained with the immune globulin and ovalbumin, since these illustrated two distinct types of behaviour, one where adsorption was high and unaffected by salt concentration, the other where adsorption was low when little salt was present but which reached a similarly high value with increasing salt concentration.

**The Effect of pH on Adsorption of Protein.**—The effect of pH on the amount of protein adsorbed onto silica gel is seen in Fig. 2. Again in this experiment 5 ml. protein solution were added to 100 mg. silica gel previously washed in the same buffer. The buffers all had ionic strengths of 0.1 and the pH after addition of the globulin as measured by the glass electrode were 4.00, 5.04, 5.98, 6.99, 7.54 and 8.34 (the ovalbumin solutions did not differ from these by more than 0.03 pH units). They had the following composition: (1) 0.45 M acetic acid + 0.10 M sodium acetate, (2) 0.04 M acetic acid + 0.10 M sodium acetate, (3) 0.070 M monosodium phosphate + 0.010 M disodium phosphate, (4) 0.019 M monosodium phosphate + 0.027 M disodium phosphate,

- (5) 0.004 M monosodium phosphate + 0.032 M disodium phosphate and  
 (6) 0.1 M glycine (+ 0.01 M sodium hydroxide) + 0.1 M sodium chloride.

After 30 min. mixing the silica gel was removed by two centrifugations. Protein solutions to which no silica gel was added served as controls, and the amount of adsorption was determined by the difference in optical density at 278 m $\mu$  between the adsorbed solutions and their controls.

It may be seen in Fig. 2 that different results were obtained with immune globulin and ovalbumin. Immune globulin showed high adsorption from pH 4 to pH 7.5, while ovalbumin showed decreasing adsorption between pH 5 to 7 and very little adsorption at more alkaline reactions. It may also be noted that the greatest differences between immune globulin and ovalbumin lay in the pH range 7 to 7.5. The data suggest that a high electrostatic charge on a protein molecule prevents its adsorption to silica gel. The same relationship is also suggested by the data showing the effect of ionic strength on adsorption (Fig. 1).

**Adsorption Isotherms of Proteins.**—Data showing the relationship between the amount of protein adsorbed and the concentration of protein remaining after adsorption is complete may be obtained by two methods, one by varying the protein and the other by varying the silica gel, that is, with

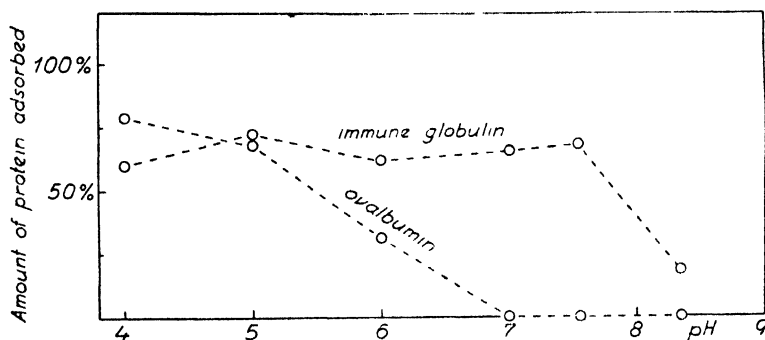


Fig. 2.—The effect of pH on the amount of protein adsorbed onto silica gel from a 0.1 % solution of the proteins indicated. The ionic strength was 0.1 throughout. Acetate buffer was used at pH 4.0 and 5.0, phosphate at pH 6.0, 7.0 and 7.7, and glycine at 8.5.

constant silica gel or with constant protein. Identical results by the two methods would be obtained only when the protein preparation was completely homogeneous. The constant protein method seemed to yield information more useful to our purposes, since with inhomogeneous protein preparation the constant silica gel method gave falsely idealized results. For example, the serum albumin preparation appeared to contain two components, one which was adsorbed very strongly and one which was adsorbed not at all under the conditions employed, and when this preparation was studied by the constant silica gel method the results showed an increasing amount of protein adsorbed in nearly exact proportion to the amount of protein remaining in solution. When the constant protein method was used the results seen in Fig. 3 c were obtained.

The experiments were carried out much as those described before. 5 ml. 0.1 % protein solution were added to a weighed amount of silica gel, the suspension was then rotated 30 min., the silica gel removed by two centrifugations, and the concentration of protein determined at 278 m $\mu$ , the amount of protein adsorbed being estimated by subtraction from the value of the control to which no silica gel had been added. The amounts of silica gel used were 5, 10, 20 (or 25), 50, 100 and 200 mg. The concentration of protein has been expressed in terms of optical density units as obtained with the Beckman spectrophotometer, since in this form it is more easily correlated with the chromatographic curves shown below. The value given for adsorption per gram was calculated by multiplying the difference between optical density of adsorbed solution and control by

5 (ml.) and dividing the product by the weight of silica gel which had been used.

The results of Fig. 3 again show the immune globulin and the ovalbumin at two extremes with the serum albumin and the serum globulin lying in between. The immune globulin showed high adsorption in the absence of sodium sulphate and the amount of adsorption was not increased by the addition of sodium sulphate. The ovalbumin, on the other hand, showed no significant adsorption except in the presence of sodium sulphate. The serum globulin showed some increase in adsorption on addition of sodium sulphate. The serum albumin showed considerably increased adsorption with sodium sulphate.

The exact course of the curves close to the origin has not been determined but it is obvious that the amount of adsorption per gram rose very steeply with low protein concentrations. The curves showing adsorption of the serum albumin in the absence of sodium sulphate show an unusual position. This

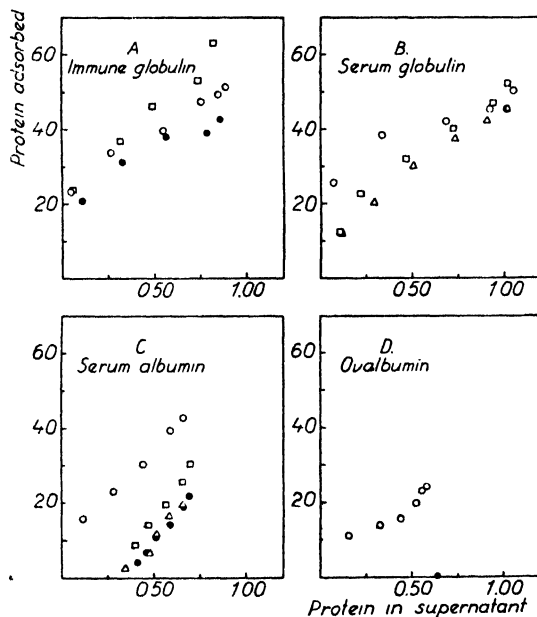


FIG. 3.—Adsorption isotherms obtained by the constant protein method. The protein concentrations after adsorption are plotted against the amount of protein adsorbed per gram of adsorbent. The salts used were sodium phosphate ( $\mu=0.1$ ) at pH 7.0 ( $\square$ ), pH 7.3 ( $\Delta$ ) and pH 7.7 ( $\bullet$ ), and the same pH 7.7 buffer plus sodium sulphate ( $\circ$ ).

presumably arises from the experimental approach (constant protein and different amounts of silica gel) and the inhomogeneity of the preparation, a minor component being strongly adsorbed whereas the major component shows very little adsorption. This supposition is borne out by the chromatogram of this preparation. It should be noted that the irregularities in the values corresponding to high concentration of protein in the supernatant arise from the method used and reflect the uncertainty in estimation of small differences between adsorbed solution and control.

**Column Experiments.**—The column experiments reported were done with frontal analysis, whereby a solution of protein is passed through the column until adsorption is complete, and the effluent contains as much of the material being adsorbed as does the solution entering the column. Although each component is not isolated separately, and after the first component each succeeding component must be contaminated with the preceding, such a procedure proved very helpful in preliminary orientation.

The column was filled with a mixture containing equal parts by weight of Supercel and silica gel prepared as described above. The filling was accomplished by preparing a thin paste of the Supercel and silica gel by adding to the dry powder enough of the solvent (solution without the protein) to yield a paste from which the small bubbles produced by mixing could rise to the surface within a few seconds. A column with high resistance to flow appeared usually to have been caused by the accidental inclusion of air bubbles in the adsorbent. It was observed that rapid flow-rates obscured the development of steps and a rate of one drop in 50 to 60 sec. was eventually adopted. Such a slow rate made it necessary for 24 to 48 hr. to elapse before the experiment was concluded. Under such conditions bacterial contamination appeared in the solution in the reservoir and in the collected effluents. To prevent the disturbances arising

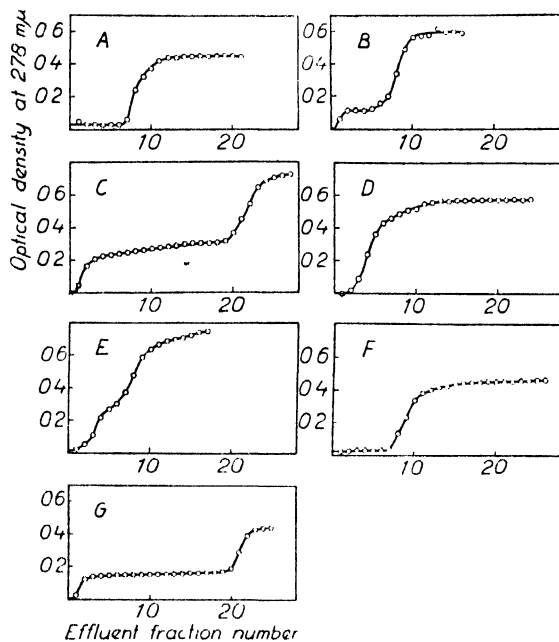


FIG. 4.—Frontal analysis chromatograms of several protein preparations and mixtures of them. The solvent was pH 7.0 sodium phosphate,  $\mu = 0.1 + 0.1$  % formalin in all cases except D and E, where it was pH 7.7 sodium phosphate,  $\mu = 0.1 + 0.5$  M sodium sulphate + 0.1 % formalin. A. 0.05 % immune globulin. Filter volume 250  $\pi$ . B. 0.05 % immune globulin + 0.05 % serum albumin. Filter volume 250  $\pi$ . C. 0.05 % immune globulin + 0.05 % serum albumin. Filter volume 500  $\pi$ . D. 0.1 % serum albumin. Filter volume 250  $\pi$ . E. 0.05 % immune globulin + 0.05 % serum albumin. Filter volume 250  $\pi$ . F. 0.1 % fetuin. Filter volume 500  $\pi$ . G. 0.05 pea-seed globulin. Filter volume 500  $\pi$ .

from bacterial growth 0.1 % formalin was incorporated in the solution. Little effect on adsorption appeared to result, and adsorption isotherms of the immune globulin on silica gel in pH 7 sodium phosphate,  $\mu = 0.1$ , with and without 0.1 % formalin were nearly identical.

Not much pressure was needed to achieve the flow-rates desired, and accurate control of the pressure was necessary. The effluent was collected in the fraction collector in successive  $3\frac{1}{2}$  ml. amounts and its protein content estimated by measurement of optical density at 278 mμ. A measurement was also made at 320 mμ in order to gain an impression of the amount of adsorbent or other interfering material which might be present. The spectrophotometric measurements were done with the solvent in the control cell. Spectroscopic analysis from 240 to 370 mμ was then carried out on samples from representative portions of the chromatogram.



Some of the chromatograms are given in Fig. 4. Most of the experiments were carried out in sodium phosphate, with a proportion of disodium to monosodium phosphate of 27/19,  $\mu = 0.1$  and pH 6.99. 0.1 % formalin was also added.

Fig. 4 A shows that when 0.05 % of immune globulin was introduced at the top of the column, the effluent first contained no protein but that after 7 volumes or 24 ml. ( $7 \times 3\frac{1}{2}$  ml.) protein began to appear and that after a total of 12 volumes the adsorbent had become saturated and the effluent contained globulin in the same concentration as the solution being introduced (the optical density of the control at 278 m $\mu$  was 0.467). On the other hand, when the serum albumin in 0.05 % concentration was studied under the same conditions the effluent showed nearly maximal protein even in the first volume. With the serum albumin the optical density of the effluent at 278 m $\mu$  never reached that of the original solution, but instead rose to a value about 80 % even when the experiment was continued for 48 hr. This indicated the presence of a component responsible for about 20 % of the optical density which was very strongly adsorbed, in confirmation of the impression gained in work reported earlier in the paper.

When a mixture of 0.05 % serum albumin and 0.05 % immune globulin was studied the pattern seen showed two distinct components as in Fig. 4 B and 4 C. In 4 B a 250  $\pi$  filter was used and the immune globulin appeared in the 5th or 6th tube although the serum albumin had been present from the 1st tube. In 4 C a 500  $\pi$  filter was used and the appearance of the globulin was accordingly delayed although more so than was expected from 4 B, since it did not appear until the 20th tube. The identity of the components in the chromatogram in 4 C was confirmed by precipitation with ammonium sulphate solutions. Aliquots from various tubes were mixed with 4 M ammonium sulphate to give 2 M and 3 M solutions. Thus in 3 M ammonium sulphate precipitate was obtained with the 5th, 10th, 15th, 20th and 25th tube, but not the 1st, whereas in 2 M ammonium sulphate precipitate was obtained with only the 25th.

The effect of increasing the salt concentration is shown in Fig. 4 D and 4 E where it is seen that in 0.5 M sodium sulphate the serum albumin adsorbs sufficiently to give a retention volume of about 10 ml. ( $3 \times 3\frac{1}{2}$  ml.). The mixture of serum albumin and serum globulin is not well resolved in 4 E where the serum albumin appeared in about the 3rd tube and the immune globulin in the 6th. The identity of the components was again checked with ammonium sulphate. 3 M ammonium sulphate gave precipitate with material from the 5th, 10th, and 15th tube but not the 1st, whereas the 2 M salt produced precipitate with the 10th and 15th, but not the 1st and 5th.

Ovalbumin also showed adsorption in column experiments in the presence of salt, and in one experiment, the tracing of which is not given here, showed a retention volume of 20 ml. ( $6 \times 3\frac{1}{2}$  ml.) with 0.1 % ovalbumin in pH 7.7 sodium phosphate,  $\mu = 0.1 + 0.75$  M sodium sulphate + 0.1 % formalin (pH 7.7 before addition of sodium sulphate), and a 250  $\pi$  filter.

Chromatograms of the serum globulin preparation indicated the preparation to be heterogeneous since the curve rose more or less linearly from the origin to a maximal value in about the 10th tube.

In Fig. 4 F may be seen the chromatogram obtained with 0.1 % fetuin, which shows only one component with a retention volume of about 26 ml. ( $8 \times 3\frac{1}{2}$  ml.). In Fig. 4 G is given the pattern of a preparation of pea-seed globulin obtained from C.-E. Danielsson. Two components are seen, but only the second appeared to be protein since the first component showed a spectrophotometric curve with a maximum at 260 m $\mu$ . Other evidence suggests that the poorly adsorbing component is a nucleic acid, or related product,\* but not enough of the material resulted from this experiment to identify it. The two seed globulins, legumin and vicilin, were apparently both contained in the second chromatographic component.

Several samples of human serum were studied by this method. The pattern seen in Fig. 5 is representative of the results obtained and is instructive because

\* C.-E. Danielsson, personal communication.

the serum contained agglutinins for *Brucella abortus*. The results of precipitation with 2 M and 3 M ammonium sulphate solutions are also presented. The serum was diluted 1/77 times in pH 7 sodium phosphate,  $\mu = 0.1 + 0.1$  % formalin and the solution showed an optical density at 278 m $\mu$  of 1.27. The results considered together show the albumin appeared soon and by the 3rd tube was near its maximum. Thereafter a small inhomogeneous component appeared from about the 5th to the 10th tubes; it did not appear to be precipitable with 3 M ammonium sulphate. After the 10th tube the globulin began to appear and increased in amount up to the 20th tube. The brucella agglutinins were first detected in the 15th tube and increased thereafter. A strongly adsorbed component, which did not appear in the eluate, was responsible for the difference between the highest reading on the eluate (1.15) and that of the unadsorbed solution (1.27). Although the experiment does not appear to yield novel information regarding the composition of human serum, it does illustrate some of the possibilities of the chromatographic analysis of complex biological mixtures and the advantages that the operator has in having the fractions separated in test-tubes.

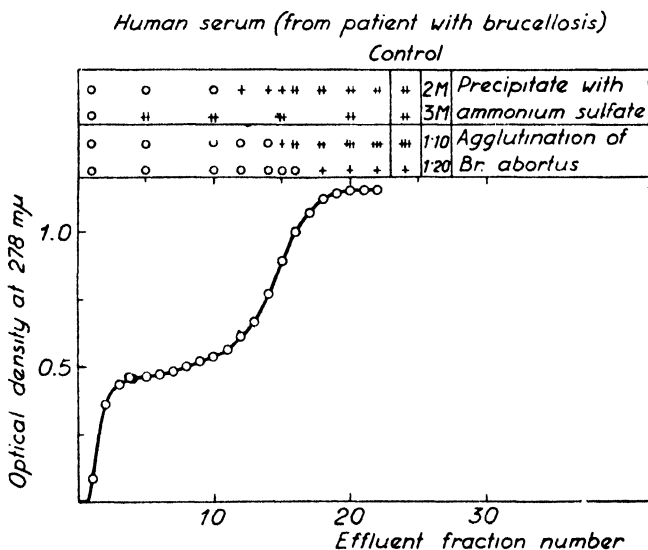


FIG. 5.—Frontal analysis chromatogram of human serum containing antibodies for *Brucella abortus*. Solvent: pH 7.0 sodium phosphate = 0.1 + 0.1 % formalin. Filter volume 500  $\pi$ .

**Comparison with other Adsorbents.**—A few experiments using other adsorbents were carried out. It has already been stated that paper and starch showed no detectable adsorption in batch experiments. For example, in one experiment 5 ml. 0.1 % serum albumin in 2 M ammonium sulphate were treated with 0.5 g. filter paper and another 5 ml. with 0.5 g. potato starch and no detectable adsorption occurred. Under the same conditions over 93 % of the serum albumin was adsorbed on silica gel.

Aluminium hydroxide was also inactive. In experiments, in which 5 ml. quantities 0.1 % ovalbumin and 0.1 % immune globulin in pH 7 sodium phosphate,  $\mu = 0.1$ , or in pH 7.2 sodium phosphate,  $\mu = 0.1 + 0.5$  M sodium sulphate, were treated with 100 mg. aluminium hydroxide (powd., Bakers' Analyzed), for 30 min., no detectable adsorption occurred.

Supracel and carbon (Norit FHX Special) were tested and found to adsorb proteins under similar conditions. Quantitative results were not achieved because of difficulties encountered in removing the adsorbent, but comparison with

results with silica gel indicated that in terms of adsorption per gram of adsorbent the carbon adsorbed two to three times as much protein as did silica gel, whereas the Supercel adsorbed only 1/10 to 1/20 times as much protein as did silica gel. With both carbon and Supercel the presence of 0.5 M sodium sulphate increased adsorption of ovalbumin and serum albumin but not of immune globulin. A few column experiments with these adsorbents have also been done with results that also show the high adsorptive capacity of carbon and low adsorption of Supercel.

### Discussion

In the work reported here some of the factors affecting adsorption of proteins to silica gel have been investigated and the role the factors play in chromatograms has been studied. It was seen in the range of ionic strength from 0.025 to 3.1 that the adsorption of the serum albumin and ovalbumin preparation increased with increasing ionic strength, while the adsorption of the serum globulin and immune globulin preparation was not much affected by ionic strength. The maximal amount of adsorption of the albumins and globulins studied was about the same, but with the albumins maximal adsorption was seen only with high salt concentrations. The difference in adsorption of albumin on the one hand and globulin on the other was greatest in the presence of salt of low ionic strength.

The effect of pH on adsorption was also different for ovalbumin and immune globulin. With ovalbumin, high adsorption was observed at pH 4 and 5 and adsorption decreased rapidly above pH 5 until it was scarcely measurable at pH 7 and above. The immune globulin behaved differently in that high adsorption was manifested from pH 4 to pH 7.5. The greatest difference between ovalbumin and immune globulin was thus seen in the range of pH 7.0 to some point below pH 8.3.

The relationships between salt concentration and pH and adsorption show that the optimal conditions for separation of albumin from globulin are a low ionic strength, of 0.1 or less, and a pH of about 7 to 8. It would also be suggested that for the separation of globulins from one another a higher pH might be needed, and that for the chromatographic separation of albumins from one another a lower pH or higher ionic strength would be of advantage.

That the electrokinetic potential of the protein molecule is of great importance in determining its adsorption to silica gel is indicated by the ionic strength—pH relationships. That this could be a concept of some functional value is illustrated by the results reported by Delbrück with a bacteriophage and sintered-glass filters,<sup>5</sup> by Reiley with Rous chicken sarcoma virus and celite,<sup>6</sup> by Davenport and Horsfall with PVM virus and lung particles,<sup>7</sup> by Reiley *et al.* with melanized particles from mouse melanomas and celite,<sup>8</sup> and by Leyon with murine encephalomyelitis and paper.<sup>9</sup> In all these cases elution was successful with distilled water or very weak salt solution, while adsorption had taken place from salt solutions of not more than physiological concentration.

The adsorption isotherms showed in general a steep rise with low protein concentrations. A shape such as this is helpful for obtaining sharp fronts but does not indicate satisfactory conditions for elution analysis when the same solvent is used. The isotherm curves were of aid in calculating the retention volume of proteins in column experiments. For example, in Fig. 4 A it could be calculated that the protein step would appear in the

<sup>5</sup> Delbrück, *J. Gen. Physiol.*, 1940, **23**, 643.

<sup>6</sup> Reiley, *Science*, 1948, **107**, 573.

<sup>7</sup> Davenport and Horsfall, *J. Expt. Med.*, 1948, **88**, 621.

<sup>8</sup> Reiley, Hesselbach, Fiala, Woode, and Burk, *Science*, 1949, **109**, 361.

<sup>9</sup> Leyon, *Arkiv Kemi* (in press).

8th or 9th tubes, and in Fig. 4 D in the 4th or 5th tubes. (The amount of silica gel in a 250  $\pi$  filter averaged about 0.32 g.)

Chromatography was carried out in columns containing silica gel, to which Supercel was added to decrease resistance to flow. With a solvent containing sodium phosphate at an ionic strength of 0.1 and pH 7.0, frontal analysis distinctly separated serum albumin and globulin. The patterns obtained with human serum illustrate some of the possibilities of the method, and its applicability to the study of biologically active materials.

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## THE APPLICATION OF CHROMATOGRAPHY TO AMINO ACIDS AND PEPTIDES

BY TUDOR S. G. JONES

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An account is given of the application of the chromatographic methods, adsorption, ion exchange and partition chromatography in its forms using paper and columns of silica gel, starch and kieselguhr to amino acids and polypeptides. Paper chromatography is considered in greater detail because of its wide application in this field. Attention is given to the conditions employed, including the influence of the paper support, the solvent systems used and their direction of flow, and to methods of detection. The use of kidney *D*-amino acid oxidase for the determination of configuration is described in greater detail than hitherto. A discussion of the mechanism of the method is followed by many examples of specific applications.

The following account of the application of the chromatographic method to the separation, identification and determination of the amino acids and peptides includes work published up to July, 1949. Not every article which has appeared is cited, and where no reference is given, the statement is derived from the author's own unpublished work. The subject will be treated under three divisions: (i) adsorption chromatography, (ii) ion exchange, (iii) partition chromatography, although no implication is made that these divisions are mutually exclusive or that the mechanisms involved follow strictly the title of the division. The following authors have published reviews mentioning amino acids and peptides in the following divisions: all methods, Martin and Synge,<sup>1</sup> Cannan,<sup>2</sup> Strain<sup>3</sup>; ion exchange, Tompkins,<sup>4</sup> Applezweig,<sup>5</sup> Kunin<sup>6</sup>; paper partition, Consden<sup>7</sup>; adsorption, Tiselius.<sup>8</sup>

<sup>1</sup> Martin and Synge, *Advances in Protein Chemistry*, II, 1.

<sup>2</sup> Cannan, *Ann. N.Y. Acad. Sci.*, 1946, **47**, 135.

<sup>3</sup> Strain, *Anal. Chem.*, 1949, **21**, 75.

<sup>4</sup> Tompkins, *J. Chem. Educ.*, 1949, **26**, 32, 92.

<sup>5</sup> Applezweig, *Ann. N.Y. Acad. Sci.*, 1948, **49**, 295.

<sup>6</sup> Kunin, *Anal. Chem.*, 1949, **21**, 87.

<sup>7</sup> Consden, *Nature*, 1948, **162**, 359.

<sup>8</sup> Tiselius, *Kolloid-Z.*, 1943, **105**, 101.

**1. Adsorption Chromatography.**—The conditions under which amino acids are adsorbed from solution, using the term in its classical sense, were defined as early as 1917,<sup>9</sup> but little advantage was taken of this discovery for the analytical separation of amino acids until the work of Tiselius<sup>10 11</sup> laid the foundations for the use of charcoal, particularly as a component for his group separation<sup>12</sup> (see §2). At about the same time a number of workers used activated alumina as the adsorptive material, but the applications belong more properly to the next section. This material has been used, however, for separation of tryptophane, tyrosine and phenylalanine.<sup>13</sup> The adsorptive chromatography of coloured derivatives of amino acids, using basic zinc carbonate,<sup>14</sup> has been taken up by Reich, who used alumina to separate small quantities of a few amino acids in the form of their *N*-azobenzene-*p*-sulphonyl<sup>15</sup> or *N*-2 : 4-dinitrophenyl<sup>16</sup> (DNP) derivatives of their methyl esters. Sanger<sup>17</sup> has used the partition column, described in §3 (a), for end-group analysis, which employs the DNP amino acids, and it is generally considered that the silica gel used functions largely as an adsorptive medium. This method has been used to determine the free amino groups in gramicidin S,<sup>18</sup> in insulin,<sup>17</sup> in salmine,<sup>19</sup> and in polymyxin D.<sup>21</sup> The use of a mixture of Lloyd's reagent and kieselguhr permitted the separation of basic and neutral amino acids.<sup>20</sup>

**2. Ion-exchange Methods.**—Ion-exchange materials have, with few exceptions, only recently been applied to amino-acid separations. Folin used natural, and later, synthetic zeolites to remove bases from biological media and Whitehorn<sup>21</sup> showed that passage of the solution through a column of the zeolite was more effective than simple agitation with it, since reversal by the liberated sodium ions was prevented. Thus originated ion-exchange chromatography, but little application was made to amino-acid separations. Titania gel<sup>22</sup> found a limited use but acid-washed alumina has been used in a number of different applications. The ionizable chloride groups, introduced by washing alumina with HCl, may be exchanged by the anions of dicarboxylic amino acids in solution in water or 80 % alcohol.<sup>23</sup> Glutamic acid may be eluted by acetate buffer and aspartic acid subsequently by caustic soda, a process difficult to understand. "Basic alumina," an analogous cation-exchange material, has been applied to the separation of basic amino acids. In the presence of formaldehyde, the neutral amino acids are rendered more acidic and the carboxyl group is strong enough to exchange with the Cl<sup>-</sup> ions of acid-washed alumina. Lederer and Tchen<sup>24</sup> found that di- and tri-peptides, glycine, serine and  $\beta$ -alanine but not  $\alpha$ -alanine were retained from a mixture with other amino acids in the presence of formalin. Acid peptides are adsorbed from water, glycyl peptides from 1 % formalin and other neutral peptides from 10 % formalin, while the adsorption of aromatic neutral peptides on carbon completes the group separation.<sup>25</sup>

<sup>9</sup> Abderhalden and Fodor, *Fermentforsch.*, 1917, **2**, 74.

<sup>10</sup> Tiselius, *Arkiv. Kemi, Min. Geol. B*, 1941, **15**, No. 6.

<sup>11</sup> Tiselius, *Science*, 1941, **94**, 145.

<sup>12</sup> Tiselius, Drake and Hagdahl, *Experientia*, 1947, **3**, 21.

<sup>13</sup> Schramm and Primosigh, *Ber. B*, 1943, **76**, 373.

<sup>14</sup> Karrer, Keller and Szönyi, *Helv. chim. Acta*, 1943, **26**, 38.

<sup>15</sup> Reich, *Biochem. J.*, 1948, **43**, xxxviii.

<sup>16</sup> Reith, *ibid.*, 1949 (in press).

<sup>17</sup> Sanger, *ibid.*, 1945, **39**, 507.

<sup>18</sup> Sanger, *ibid.*, 1946, **40**, 261.

<sup>19</sup> Tristram, *Nature*, 1947, **160**, 637.

<sup>20</sup> Bergdoll and Doty, *Ind. Eng. Chem. (Anal.)*, 1946, **18**, 600.

<sup>21</sup> Whitehorn, *J. Biol. Chem.*, 1923, **56**, 751.

<sup>22</sup> Johnston, cited by Cannan.<sup>2</sup>

<sup>23</sup> Turba and Richter, *Ber. B*, **75**, 1942, 340. Wieland, *ibid.*, 1901. Wieland, *Naturwiss.*, 1942, **30**, 374.

<sup>24</sup> Lederer and Tchen, *Biochim. Biophys. Acta*, 1947, **1**, 35.

<sup>25</sup> Jutisz and Lederer, *Nature*, 1947, **159**, 445. Dubnoff, *J. Biol. Chem.*, 1941, **141**, 711. Archibald, *J. Biol. Chem.*, 1944, **156**, 121. Freudenberg, Walch and Moltor, *Naturwiss.*, 1942, **30**, 87.

The introduction of anion-exchange resins of high capacity has interested a number of workers in this field,<sup>26</sup> who have presented detailed theoretical<sup>2</sup> and practical studies. Consden, Gordon and Martin<sup>27</sup> adopted the principle of establishing a constant pH value in finely ground Amberlite IR-4 and in this way were able to effect a complete separation of glutamic, aspartic and cysteic acids from a wool hydrolyzate. These acidic amino acids were first separated from the neutral and basic acids on a column at pH 3 to 4, eluted in small volume by displacement by N HCl, placed on a column already brought to pH 2.5 by washing with 0.003 N HCl and developed with the same solution. The recoveries were not quantitative. These results are interpreted as involving a depression of the iso-electric point of the acids when adsorbed, since the optimum pH for separate elution should be pH 4.5, at which pH the acids are actually retained. The same resin was used by Drake,<sup>28</sup> who eluted the glutamic acid with 0.05 % acetic acid and aspartic acid with N HCl. An automatic sampling device for applying small portions of the effluent to paper for ninhydrin reaction and matching enabled quantitative determinations to be made.

Cation-exchange resins were originally applied to separate basic from neutral amino acids in analysis, but difficulties were evident as it was not appreciated that the neutral acids have a distinct affinity for the sulphonic groups of the resins used.<sup>2</sup> With the intention of developing preparative rather than analytical methods, Partridge and Westall<sup>29</sup> have investigated the behaviour of Zeo-Karb 215, a sulphonic acid resin. In this method all the amino acids are adsorbed on the acid resin and, in order to avoid the difficulties of selective elution, the displacement technique of Tiselius and Claesson<sup>30</sup> was employed. Changes in the composition of the effluent were measured by a combination of methods: measurement of conductivity or pH, paper chromatography or measurement by titration of small successive fractions. A detailed consideration is given of many of the factors involved. The retention isotherms for single amino acids take the form of the Langmuir equation. Effective separation is achieved if the iso-electric points of the components differ by more than 0.5 to 1.0 pH unit. Use is made of the graphical method of Tiselius<sup>31</sup> for calculating the concentrations of a given displacing agent necessary for a given concentration of the issuing amino acid. In a subsequent paper<sup>32</sup> we are promised data on the separation of complicated mixtures including protein hydrolyzates.

Perhaps the most valuable feature of the ion-exchange methods is the scale on which they may be applied, and there is no reason why this may not be increased to full plant operation. An application of this sort is the isolation of the polypeptide antibiotics, the polymyxins, from the crude culture fluid in which they are elaborated.<sup>33</sup> Another application in this field was the separation of the amino acids shown to be present in polymyxin A (Aerosporin).<sup>34</sup> This led to the isolation of an unknown amino acid retained on Zeo-Karb 215 in the presence of ammonia and displaced by sodium hydroxide, and of obviously basic character. The attempted isolation of  $\gamma$ -aminobutyric acid, found by paper chromatograms to be present in molasses, using the carboxylic cation exchanger Zeo-Karb 216, was not successful.<sup>35</sup> This may have been due to the effect of large concentrations of sugar on the pK of the amino acid comparable with the effects of acetone.<sup>12</sup>

Esters of polysaccharides with polybasic acids, such as cotton succinate or phthalate, have been suggested for use as carboxylic cation exchangers.<sup>36</sup>

<sup>26</sup> Turba, Richter and Kuchar, *ibid.*, 1943, **31**, 508. Englis and Fiess, *Ind. Eng. Chem.*, 1944, **36**, 604. Cleaver, Hardy and Cassidy, *J. Amer. Chem. Soc.*, 1945, **67**, 1343. Cannan, *J. Biol. Chem.*, 1944, **152**, 401.

<sup>27</sup> Consden, Gordon and Martin, *Biochem. J.*, 1948, **42**, 413.

<sup>28</sup> Drake, *Nature*, 1947, **160**, 602.

<sup>29</sup> Partridge and Westall, *Biochem. J.*, 1949, **44**, 418.

<sup>30</sup> Tiselius and Claesson, *Arkiv Kemi, Min. Geol. B*, 1942, **15**, No. 18.

<sup>31</sup> Tiselius, *ibid. A*, 1943, **16**, No. 18.

<sup>32</sup> Partridge, *Biochem. J.*, 1949, **44**, 521.

<sup>33</sup> Jones, Johnston and Hood, *Brit. Pat. Appl.* 1994/49.

<sup>34</sup> Jones, *Biochem. J.*, 1948, **42**, xxxv.

<sup>35</sup> Jones (unpublished observations).

<sup>36</sup> McIntire and Schenk, *J. Amer. Chem. Soc.*, 1948, **70**, 1193.

The capacity of these materials can never be very high, but the properties of a material of a form familiar as cotton wool, and its relative insolubility, render it worthy of trial on very small-scale separations.

The separation of amino-acid mixtures into groups for subsequent analysis has been achieved by the methods outlined in these two sections. The use of acid alumina with formaldehyde<sup>37</sup> and the use of a hydrogen-exchange resin by Block<sup>37</sup> to separate basic from neutral amino acids have been described in principle already and systems are available<sup>38, 39</sup> by which four groups may be obtained. For analytical purposes, more specific separations may be achieved by partition methods, without group separations, but comparative investigation of the two methods remains to be done.

**3. Partition Chromatography.**—(a) SILICA GEL AND DIATOMACEOUS EARTH. —Taking advantage of the gradation in partition coefficients of the acetyl amino acids, Martin and Synge<sup>39</sup> devised a counter-current machine for their separation, and used it in the analysis of hydrolyzates of wool. This idea was soon modified to form the well-known partition chromatogram,<sup>40</sup> and a scheme of analysis for certain acetyl amino acids was devised.<sup>41</sup> This was modified later by Tristram.<sup>41a</sup> The method has been used for the analysis of hydrolyzates of wool,<sup>40, 41</sup> gelatin,<sup>41, 42</sup> gramicidin,<sup>43, 44</sup> tyrocidine,<sup>41</sup> myosin and fibrin,<sup>45</sup> gramicidin S,<sup>46</sup> methylated proteins,<sup>47, 48</sup> and salmine.<sup>19</sup> The preparation of silica gel of the non-adsorptive nature necessary for the method has not proved to be straightforward,<sup>19</sup> and this is one factor which has tended to prevent its rapid development. Difficulties in the preparation of silica gel suitable for separating DNP-amino acids are overcome by the use of buffered columns<sup>49</sup> according to Blackburn,<sup>50</sup> who specifies solvents suitable for separating different groups of DNP-acids.

Diatomaceous earth has been used as a substitute for silica gel<sup>51</sup> and has found application in the analogous problem of the separation of the penicillins.<sup>52</sup> Its application to the determination of *N*-dinitrophenyl derivatives of amino acids in hydrolyzates combines the use of a separation technique with the possibilities of a sensitive absorptiometric method. It has been so applied to the determination of both free amino groups and the individual amino acids in polymyxin *D*.<sup>51</sup> Wieland and Fremery<sup>51a</sup> have described the use of partition columns of silica gel for the separation of the coloured copper complexes of amino acids. The amino groups are in combination with the copper atoms and adsorption is absent.

(b) STARCH CHROMATOGRAPHY.—A development of paper partition chromatography, which is treated in the next sections, that is, chromatography on starch, was introduced in order to increase the scale on which free amino acids may be separated.<sup>53, 54</sup> In the hands of Moore and Stein<sup>53, 54, 55</sup> it has been converted into a tool for the complete separation and determination of the amino acids in

<sup>37</sup> Block, *Proc. Soc. Exp. Biol. N.Y.*, 1942, **51**, 252.

<sup>38</sup> Fromageot, Jutisz and Lederer, *Biochim. Biophys. Acta*, 1948, **2**, 487.

<sup>39</sup> Martin and Synge, *Biochem. J.*, 1941, **35**, 91.

<sup>40</sup> Martin and Synge, *ibid.*, 1358.

<sup>41</sup> Gordon, Martin and Synge, *ibid.*, 1943, **37**, 79, 313; 1944, **38**, 65.

<sup>41a</sup> Tristram, *ibid.*, 1944, **38**, 721.

<sup>42</sup> Gordon, Martin and Synge, *ibid.*, 1943, **37**, 92.

<sup>43</sup> Gordon, Martin and Synge, *ibid.*, 1943, **37**, 86.

<sup>44</sup> Synge, *ibid.*, 1944, **38**, 285.

<sup>45</sup> Bailey, *Advances in Protein Chemistry*, 1945, **1**, 289.

<sup>46</sup> Synge, *Biochem. J.*, 1945, **39**, 363.

<sup>47</sup> Gordon, Martin and Synge, *ibid.*, 1943, **37**, 538.

<sup>48</sup> Blackburn, Consden and Phillips, *Biochem. J.*, 1944, **38**, 25.

<sup>49</sup> Levi, *ibid.*, 1948, **43**, 257.

<sup>50</sup> Blackburn, *Nature*, 1949, **163**, 955.

<sup>51</sup> Bell *et al.*, *Ann. N.Y. Acad. Sci.*, 1949, **51**, 897.

<sup>51a</sup> Wieland and Fremery, *Ber. B.*, 1944, **77**, 234.

<sup>52</sup> Elsdon and Synge, *Biochem. J.*, 1944, **38**, ix.

<sup>53</sup> Moore and Stein, *Ann. N.Y. Acad. Sci.*, 1948, **49**, 265.

<sup>54</sup> Stein and Moore, *J. Biol. Chem.*, 1948, **176**, 337, 367.

<sup>55</sup> Moore and Stein, *ibid.*, 1949, **178**, 53.

a protein hydrolyzate. Indeed, the much analyzed proteins,  $\beta$ -lactoglobulin and bovine serum albumin, have been examined by the method with results comparable with the best recorded.<sup>56</sup> Another example of its use was the separation, on a considerable scale from a variety of sources, of a peptide,<sup>57</sup> thought to be reasonably homogeneous. The amino-acid composition of this peptide was also determined by this method. Another application was the demonstration of the conversion of lysine to  $\alpha$ -amino-adipic acid by guinea-pig liver.<sup>58</sup> The disadvantage of the starch column is its exceedingly slow rate of flow and means must be provided for collecting many small fractions of equal volume for the quantitative application.

(c) CONDITIONS OF PAPER CHROMATOGRAPHY.—The theory of paper partition chromatography is covered by other papers in this Discussion, but it is appropriate to treat here those aspects special to amino acids and peptides and to describe findings which may have a bearing on the method as a whole. The problems involved in the paper chromatography of amino acids, for which the method was originally introduced,<sup>60</sup> namely, the choice of paper and solvent, the methods of recognition and determination and the effects of physical conditions, are the same problems as are met with in the examination of other classes of compounds. The choice of paper is limited to what is available commercially, and little modification is possible apart from washing to remove undesirable contaminants, or impregnation with such substances as buffering materials or even hydrophobic substances such as rubber.<sup>61</sup> In general, a given grade of paper behaves uniformly, except for occasional unexplained variations, which cannot be ascribed to any known cause except the lack of uniformity of the paper batch. A conspicuous example is the variation<sup>62</sup> in the intensity of fluorescence<sup>63</sup> of amino acids, even on consecutive sheets of paper drawn from a single batch. The explanation of this lies in the practice of the manufacturer of bulking the output of a number of cutting machines from a number of different rolls of paper. It is thus possible that a single batch may incorporate paper originating from several different batches of raw material, without influence on the mechanical properties of the paper as filter paper but having an important effect on the properties needed for a chromatographic support. The simple solution is to specify paper for chromatography and a leading manufacturer, we are assured, will see that a given batch at least comes from one source of raw material. For uniformity, the direction of flow of the solvent relative to the direction of cut of the paper<sup>64</sup> is important. With rectangular sheets, this point scarcely arises, but with square sheets a direction should be chosen following observation of the texture. Another factor in the choice of a paper is the degree of separation achieved in a given time with a given solvent. With the solvents in common use, the tendency is to use Whatman 4 paper. The texture of the paper has an influence on the separations achieved and it has been found that Whatman 7 paper gives with amino acids round spots showing less dispersion in the direction of flow.

The choice of solvents for amino acids follows that of the originators<sup>60</sup> of the method, with few modifications, the commonly used solvents for two-dimensional work being the collidine fraction of coal tar distillate, and phenol. The collidine fraction is a mixture and, if pure collidine and lutidine are available, equal parts of these<sup>66</sup> will reproduce the standard chromatogram maps published.<sup>60-65</sup> The addition of ammonia to phenol causes the basic amino acids to take up positions on the two-dimensional chromatogram well removed from the acidic acids.<sup>61</sup> Alkaline phenol tends to oxidize easily,

<sup>56</sup> Stein and Moore, *J. Biol. Chem.*, 1949, **178**, 79.

<sup>57</sup> Borsook, Deasy, Haagensmit, Keighley and Lowy, *ibid.*, 1949, **179**, 705.

<sup>58</sup> Borsook, Deasy, Haagensmit, Keighley and Lowy, *ibid.*, 1948, **173**, 424.

<sup>59</sup> Randall and Martin, *Biochem. J.*, 1949, **44**, ii.

<sup>60</sup> Consden, Gordon and Martin, *ibid.*, 1944, **38**, 224.

<sup>61</sup> Boldingh, *Experientia*, 1948, **4**, 270.

<sup>62</sup> Woiwod and Jones (unpublished).

<sup>63</sup> Phillips, *Nature*, 1948, **161**, 53.

<sup>64</sup> Bull, Hahn and Baptist, *J. Amer. Chem. Soc.*, 1949, **71**, 550.

<sup>65</sup> Dent, *Biochem. J.*, 1948, **43**, 169.



giving dark fronts, and various expedients, such as addition of KCN, cupron, oxine and displacing the atmosphere by coal gas, have been devised to minimize the oxidation and metallic interference. Collidine interferes with the quantitative determination of amino acids by Woiwod's<sup>68</sup> method and needs periodical purification and may with advantage be replaced by butanol equilibrated with aqueous acetic acid,<sup>67</sup> different concentrations of acetic acid from 5 % to 25 % being of use for the separation of different mixtures. The overlapping of the basic amino acids with other amino acids is negligible if neutral phenol follows butanol-acetic acid in a two-dimensional chromatogram, so that the addition of ammonia becomes a disadvantage to be avoided. The addition of oxine to a good commercial grade of phenol enables clean chromatograms to be produced and renders special purification unnecessary. Workers who have standardized their chromatograms using collidine and alkaline phenol will be loath to change to different solvents, but newcomers are recommended to try both systems. It is of interest that Phillips<sup>68</sup> finds butanol-20 % acetic acid to be superior to other solvents for the peptides of an enzymic digest of insulin. Tertiary amyl alcohol in the presence of diethylamine has been found by Work<sup>122</sup> to effect the separation of leucine, isoleucine and phenylalanine.

Williams and Kirby<sup>69</sup> have described a modification of the method of Consden, Gordon and Martin,<sup>60</sup> in which the solvent is caused to move over the paper by capillary ascent. The paper, in the form of an open cylinder secured by staples along overlapping edges, is stood on end in a dish of solvent. Other modifications to this method have been described,<sup>70</sup> designed to render the paper, wet with solvent, sufficiently rigid to stand. It is here considered that nothing is simpler for two-dimensional work than the original apparatus, in one of its slight modifications described by Dent<sup>65</sup> or Woiwod,<sup>71</sup> that of the latter being very similar to that used by the author.

Upward flow may be used with advantage for narrow strips in very simple apparatus and in the test-tube.<sup>72</sup> Its use for thick paper is described in §3 (g).

(d) RECOGNITION OF AMINO ACIDS AND PEPTIDES.—The method universally adopted for the recognition of amino acids and peptides on paper is the ninhydrin reaction, the reagent being applied to the paper as a solution in dry or aqueous butanol or chloroform in the form of a spray.<sup>60</sup> The paper is then dried and the colour developed at slightly elevated temperatures for amino acids or higher temperatures up to 100° for peptides. The reaction is said to go to completion more rapidly if the final development is carried out in steam.<sup>64</sup> The reagent may be incorporated in the solvent,<sup>73</sup> but a disadvantage of this is the cost. With relatively volatile solvents, the preliminary drying of the paper may be avoided by evaporation in a stream of air to a state sufficiently dry to avoid diffusion of the spots, spraying with ninhydrin and subsequent development. The addition of small amounts of collidine<sup>71</sup> or pyridine<sup>68</sup> to the ninhydrin solution causes a differentiation of the colour of the spots as an aid to recognition, while the presence of phenol renders the colour more uniform and suitable for densitometry.<sup>64</sup> The ninhydrin method is not specific for  $\alpha$ -amino acids; on paper, positive reactions are given by many bases, although with diminished intensity, as well as by  $\beta$ - and other amino acids. The sensitivity of the ninhydrin reaction for a number of amino acids has been compared by Pratt and Auclair.<sup>68a</sup> Other methods of recognition applicable to all amino acids are the naphthoquinone- $\beta$ -sulphonate reagent,<sup>74</sup> which gives spots of less intensity than ninhydrin, and the fluorescence in filtered ultra-violet light shown by amino acids and peptides,<sup>63</sup> but which is also less sensitive than the ninhydrin method, needs careful drying of the paper and is subject to considerable variation with the

<sup>67</sup> Partridge, *Biochem. J.*, 1948, **42**, 238. Jones, *ibid.*, xxxv.

<sup>68</sup> Phillips, *Biophys. Acta*, 1949, **3**, 341.

<sup>68a</sup> Pratt and Auclair, *Science*, 1948, **108**, 213.

<sup>69</sup> Williams and Kirby, *ibid.*, 1948, **107**, 481.

<sup>70</sup> Wolfson, Cohn and Devaney, *ibid.*, 1949, **109**, 541.

<sup>71</sup> Woiwod, *J. Gen. Microbiol.*, 1949, **3**, 312.

<sup>72</sup> Rockland and Dunn, *Science*, 1949, **109**, 539.

<sup>73</sup> Nicholson, *Nature*, 1949, **163**, 954.

<sup>74</sup> Folin, *J. Biol. Chem.*, 1922, **51**, 377, 393.

paper batch. Special methods have been described for the recognition of specific amino acids, e.g., the use of *o*-phthaldehyde for glycine<sup>76</sup>; potassium iodo-platinate<sup>77</sup> and the sodium azide-iodine<sup>78</sup> reaction for sulphur-containing amino acids; treatment with periodic acid followed by Nessler's reagent for hydroxyamino acids<sup>79</sup>; diazotized sulphanilic acid for tyrosine and histidine<sup>80</sup>; *p*-dimethylamino-benzaldehyde for tryptophane<sup>7</sup>; 2:4-dinitrophenylpyridinium chloride for reduced tryptophane<sup>7</sup>; *p*-nitrobenzoyl chloride and pyridine for *N*-methylamino acids.<sup>81</sup> Iodine sublimed on to the paper or sprayed on in alcoholic solution gives temporary spots of a brown colour,<sup>84</sup> which soon fade by evaporation and lead to no interference with the subsequent use of another reagent. Another method consists in treatment of the paper with a 5 % solution in chloroform of copper acetylacetonate,<sup>85</sup> which gives a quantitative conversion of the amino acid to its copper complex. Excess of the reagent is removed by chloroform and the copper in the complex is revealed by treatment with 1 % rubenic acid in acetone. This and similar methods may be used to distinguish between  $\alpha$ -amino acids and others which give the ninhydrin reaction.

An elegant method due to Syngé<sup>86</sup> is the use of the *D*-amino acid oxidase of sheep's kidney for the determination of the configuration of amino acids on paper chromatograms. The method may be used for leucine, valine, threonine and serine but has failed with *D*- $\alpha$ -diaminobutyric acid and would presumably fail for other diamino acids. The method was applied to the valine residues of gramicidin by Syngé and for the demonstration of the presence of *D*-leucine and *L*-threonine in polymyxin *A* (Aerosporin) by Jones.<sup>86</sup> The method has lately been published by Syngé, but a description of it would find a place here. A chromatogram is prepared from a suitable quantity of the amino-acid mixture to give about 25  $\mu$ g. of the one to be tested. On the same paper are placed 25  $\mu$ g. of the *D*- and *L*-forms of the acids, and graded mixtures of them. The amino acid spots are marked by their ultra-violet fluorescence and the paper in their vicinity is sprayed with a solution of the *D*-amino acid oxidase. The damp paper is then kept for three hours in an oxygenated water-saturated atmosphere at 40°C. It is then dried in a hot room, sprayed with ninhydrin in water-saturated butanol, and the colour developed at 40°C. Amino-acid mixtures containing the *D*-form will be found to yield spots of diminished intensity. The enzyme solution is prepared according to the directions of Negelein and Brömel,<sup>87</sup> proceeding as far as their step 5. Instead of preparing the solid, the solution is dialyzed against 0.2 M pyrophosphate buffer, and the co-enzyme is added as described in the paper. This preparation gives little or no background.

(e) QUANTITATIVE PAPER CHROMATOGRAPHY.—The introduction of partition chromatography was a great stimulus to the established interest in the analysis of proteins and other nitrogenous biological products, and attempts were soon made to apply quantitative methods to the materials separated on the paper chromatogram, both *in situ* and after removal from the paper. Included in the former are visual matching of the spot formed with ninhydrin with a spot due to a known quantity of the amino acid,<sup>88 89</sup> photoelectric determination of the optical density of the coloured spot,<sup>84 89</sup> and measurement of the area of the spot.<sup>90</sup> Another *in situ* method of great sensitivity results from the incorporation

<sup>76</sup> Patton and Foreman, *Science*, 1949, **109**, 339.

<sup>77</sup> Winegard, Toennies and Block, *ibid.*, 1948, **108**, 506.

<sup>78</sup> Chargaff, Levine and Green, *J. Biol. Chem.*, 1948, **175**, 67.

<sup>79</sup> Dent, *Biochem. J.*, 1947, **41**, 240.

<sup>80</sup> Plattner and Nager, *Helv. chim. Acta*, 1948, **31**, 2203.

<sup>81</sup> Brante, *Nature*, 1949, **163**, 651.

<sup>82</sup> Wieland and Fischer, *Naturwiss.*, 1948, **35**, 29.

<sup>83</sup> Syngé, *Biochem. J.*, 1949, **44**, 542.

<sup>84</sup> Jones, *Biochem. J.*, 1948, **42**, lix.

<sup>85</sup> Negelein and Brömel, *Biochem. Z.*, 1938/9, **300**, 225.

<sup>86</sup> Polson, Mosley and Wyckoff, *Science*, 1947, **105**, 603.

<sup>87</sup> Fosdick and Blackwell, *ibid.*, 1949, **109**, 314. Block, *ibid.*, 1948, **108**, 608.

<sup>88</sup> Fisher, Parsons and Morrison, *Nature*, 1948, **161**, 764. Brimley, *ibid.*, 1949, **163**, 215.

of radioactive carbon,<sup>91</sup> sulphur<sup>92</sup> or iodine<sup>93</sup> in the amino acid or a derivative. The spot may then be detected by autoradiography<sup>94</sup> and determined quantitatively by the usual methods.<sup>95</sup> Isotopic *p*-iodobenzenesulphonyl derivatives of any amino acids may be employed.<sup>96</sup> The ninhydrin spot may be removed from the paper,<sup>97</sup> or the spot may be marked by a minimal quantity of ninhydrin and then removed,<sup>98</sup> and the coloured ninhydrin product determined absorptiometrically. Another reaction applied to amino acids removed from the paper is the formation of the copper complex by reaction with copper phosphate suspension,<sup>99</sup> which may be formed in the eluate by simple mixture of copper sulphate and  $\text{Na}_2\text{HPO}_4$  titrated to pH 10.2. The copper in the complex may then be determined absorptiometrically by one of the usual colour reactions,<sup>100</sup> or the complex itself may be determined polarographically.<sup>100 101</sup> In the first method the excess copper phosphate must be removed by filtration, but this is unnecessary in the second since insoluble materials are not reduced at the dropping mercury cathode. One advantage of the absorptiometric method is that, since the copper equivalent to the amino acid is determined, the colour produced is substantially the same for most common amino acids and mixtures such as leucine-isoleucine-phenylalanine, valine-methionine and glycine-serine which occur on single dimensional chromatograms may be determined. Collidine interferes with this method by preventing the quantitative removal of leucine,<sup>99</sup> at least, and this is another reason for its replacement by butanol-acetic acid mixture. Phenol has no deleterious effects, provided that it is properly removed by evaporation. The polarographic method determines the diffusion current of the copper complex of the amino acid and, in general, this differs for different amino acids in a manner mainly dependent upon the size of the complex, and independent of the nature of the amino acid. For example, glycine gives a higher diffusion current than leucine or lysine, which are approximately equal. All these amino acids yielded complexes, at the pH employed, containing one atom of copper to two molecules of the amino acid. In contradistinction to this,  $\alpha$ - $\gamma$ -diaminobutyric acid forms a complex with copper which contains one molecule per atom of copper; this smaller complex yields a much higher diffusion current than glycine. It was found possible to soak off the amino acids in 0.18 M  $\text{Na}_2\text{HPO}_4$  solution, and addition of an equal volume of copper phosphate suspension was all that was necessary before placing the solution in the polarograph cell. This reagent enabled a reproducible blank diffusion current to be measured, contrary to Martin and Mittelmann's<sup>100</sup> experience with the reagent of Pope and Stevens.<sup>102</sup> It has been found advantageous to attempt the elimination of variations caused during the running of the chromatogram. Block,<sup>99</sup> using a densitometric ninhydrin method, measures five consecutive strips containing the amino acids. Woiod<sup>99</sup> prepares a chromatogram of a large number of identical amounts of the mixture, placed on a starting line parallel with the shorter side of a 22 in.  $\times$  18½ in. sheet of paper. A strip is then cut so as to include the line of spots corresponding to one amino acid, determined by fluorescence, and the amino acid removed by soaking in water is reduced to dryness by evaporation under reduced pressure. The amino acid is then treated with the copper reagent.

(f) THE MECHANISM OF THE METHOD AS APPLIED TO AMINO ACIDS AND PEPTIDES.—In applying the above methods to the examination of biological preparations of single substances, the following considerations are relevant. In the first

<sup>91</sup> Calvin and Benson, *Science*, 1949, **109**, 140. Fink and Fink, *ibid.*, 1948, **107**, 253.

<sup>92</sup> Tomarelli and Florey, *ibid.*, 1948, **107**, 630.

<sup>93</sup> Taugo, Chaikoff and Tong, *J. Biol. Chem.*, 1949, **178**, 997.

<sup>94</sup> Fink and Fink, *Science*, 1948, **107**, 253. Fink, Dent and Fink, *Nature*, 1947, **160**, 801.

<sup>95</sup> Keston, Udenfriend and Levi, *J. Amer. Chem. Soc.*, 1947, **69**, 3151.

<sup>96</sup> Naftalin, *Nature*, 1948, **161**, 763.

<sup>97</sup> Landua and Awapara, *Science*, 1949, **109**, 385.

<sup>98</sup> Woiod, *Nature*, 1948, **161**, 169; *Biochem. J.* (in press).

<sup>99</sup> Woiod (private communication).

<sup>100</sup> Martin and Mittelmann, *Biochem. J.*, 1948, **43**, 353.

<sup>101</sup> Jones, *ibid.*, 1948, **42**, lix.

<sup>102</sup> Pope and Stevens, *ibid.*, 1939, **33**, 1070.

place the purity of these materials is often difficult to achieve and equally difficult to establish. When therefore faint spots appear on chromatograms, consideration must be given to the question whether they are due to components of the molecule, e.g., in the case of a protein hydrolyzate, the amino acids. A knowledge of the order of the molecular weight of the substance under investigation will enable a decision to be taken whether the faint spots observed could represent a minimum of one unit amino acid in the molecule. The finding of small amounts of material on paper chromatograms, prepared from biological mixtures in positions usually occupied by standard amino acids, should only be taken as a challenge to attempt the isolation of the material on a scale sufficient to prove its identity by the established procedures. One important result to be obtained from paper chromatograms is the demonstration of the absence, in significant amount, of a particular amino acid.<sup>108</sup> In the field of such complexity as the composition of proteins, the value of limiting the number of the components can scarcely be over-emphasized.

In their original publication, Consden, Gordon and Martin<sup>60</sup> held that the paper played the role of an inert support. Whatever the mechanism with other materials, it is certain that some adsorption of amino acids on the paper takes place. Evidence for this is found if sufficient paper is cut from a chromatogram and eluted, when amino acids which have passed over the strip will be found in the eluate. Moore and Stein<sup>63</sup> consider that with starch chromatograms adsorption plays a prominent part and cite experiments in which no immobile phase was employed and the results of which were comparable with those of partition chromatograms. The separation of the anions of amino acid and peptide salts from the cations would argue a selective 'adsorption' for the cation, and a mechanism for this is available in the carboxyl groups present in the partly degraded cellulose of the paper. The displacement of certain amino acids by slightly slower ones, present in large amount, has been ascribed to a kind of salting-out process; it could equally well be a displacement development on an ion-exchange medium. Whatever the mechanism, the separation of the salts as ions has a distinctly practical significance. Some salts such as picrates, picrolonates or double salts are very readily dissociated on the paper and bases applied as these salts are found to have separated. On the other hand, sulphates of basic amino acids as well as of other bases, such as ergometrine, are found to be moved by such solvents as butanol-acetic acid at rates much slower than the corresponding hydrochlorides, tartrates, maleates or the free bases. Free sulphuric acid in excess is found to have a high  $R_F$  value in these solvents, and it is of advantage to remove sulphuric acid or sulphates before attempting to separate basic amino acids.

Another consequence of the mechanism of the chromatographic process is the loss which occurs in the progress of the amino acid along the paper.<sup>69</sup> In many publications, this loss is not mentioned and in others it is buried in the losses consequent upon the series of operations made to form a quantitative estimate. Many methods proposed attempt to overcome this defect by a comparison of the unknown with known amounts applied to the paper at the same time. It appears that losses occur to an extent roughly proportional to the distance travelled on the paper.<sup>68</sup> It has been reported that the loss which occurs on separating metallic cations is uniform along the paper strip. This may be due to the acidic solvents employed or to a different mechanism for the separation. It is to be hoped that some method which is exact and does not involve removal from the paper will be applied to this problem to divide the possible losses into their components.

(g) THICK PAPER.—The use of paper chromatograms for preparative separations, although discussed by Consden, Gordon and Martin,<sup>60</sup> has received little development, probably because of the introduction of the starch column. Two methods are available: (i) to use many sheets of paper and (ii) to use thicker paper. The above authors mentioned Whatman Accelerator paper as a possible support, but this proved too brittle for use with descending flow. With upward flow, however, difficulties due to this cause are overcome, and reasonable one-dimensional separation of small numbers of amino acids which normally separate

<sup>108</sup> Dent and Rose, *ibid.*, 1948, 43, liv.

well can be achieved. One method is to clamp the sheet of paper at the upper edge between strips of plate glass and hang it so that the lower edge rests in the solvent in the trough. One sheet of paper will easily accommodate 20 mg. lots of each amino acid distributed along a line parallel with the lower edge, and the bands of amino acids will usually be less than 1 cm. deep.

(h) SPECIFIC APPLICATIONS: AMINO ACIDS.—The most spectacular applications of paper chromatography have been in the field of polypeptide antibiotics and among those examined in this way are the polymyxins,<sup>105</sup> gramicidin S<sup>107</sup> and the licheniformins.<sup>108</sup> The enniatins,<sup>109</sup> while not polypeptides but cyclic esters, can be discussed with this class. The amino acids present in these antibiotics have been identified and new natural ones discovered, and the proportions present and their configurations determined. The new amino acids are  $\alpha$ - $\gamma$ -diaminobutyric acid, found in polymyxin A (Aerosporin) by its characteristic position among the basic amino acids and the characteristic colour with ninhydrin,<sup>110</sup> and the N-methyl amino acids, methyl valine, methyleucine and methylisoleucine, found in the enniatins. The occurrence of more than one polypeptide antibiotic synthesized by a single strain of an organism (the licheniformins) and by different strains (the polymyxins) is revealed when their amino-acid composition is determined. The other antibiotics present, so far as is known, no unusual qualitative features, and the reported occurrence of D- and L-forms<sup>111</sup> of the same amino acid in one molecule is resolved by the discovery that the antibiotic (gramicidin) is a mixture.

The identification of free amino acids in normal<sup>112</sup> and abnormal animal tissues, including tumours,<sup>113</sup> in the secretions of normal<sup>92</sup> <sup>114</sup> and diseased mammals<sup>114</sup> <sup>108</sup> <sup>115</sup> and in the hæmolymph of insects,<sup>116</sup> serves as an introduction to the enormous scope opened out for future investigation. In the plant world, the following of amino-acid metabolism by ordinary paper chromatograms<sup>117</sup> and those obtained after introduction of radioactive tracer elements<sup>94</sup> <sup>91</sup> has been begun. The amino-acid compositions of biologically important proteins and polypeptides, including the hormone insulin,<sup>98</sup> <sup>118</sup> the enzymes lysozyme,<sup>119</sup> and streptokinase, the tissue peptide of Borsook,<sup>97</sup> the protamine salmine,<sup>120</sup> and the fibrous proteins wool and silk,<sup>98</sup> have all been recently examined by this means.

In the study of bacteria, the smallness of the sample necessary for examination will prove to be the decisive factor in the development of qualitative and quantitative methods. Already studies have appeared on the occurrence of free amino acids in bacterial cells,<sup>121</sup> <sup>122</sup> on the amino-acid composition of hydrolyzates,<sup>121</sup> <sup>123</sup> <sup>123</sup> on the amino-acid changes in the medium during growth, including uptake and secretion,<sup>124</sup> and the composition of bacterial products such as diphtheria toxin and toxoid<sup>125</sup> and, of course, the antibiotics mentioned

<sup>105</sup> Jones, *Ann. N.Y. Acad. Sci.*, 1949, **51**, 909.

<sup>107</sup> Consden, Gordon, Martin and Synge, *Biochem. J.*, 1947, **41**, 596.

<sup>108</sup> Work (private communication).

<sup>109</sup> Plattner and Nager, *Helv. chim. Acta*, 1948, **31**, 2192.

<sup>110</sup> Catch and Jones, *Biochem. J.*, 1948, **42**, lii.

<sup>111</sup> Christensen, *J. Biol. Chem.*, 1943, **151**, 319; 1944, **154**, 427. Christensen and Hegsted, *ibid.*, 1945, **160**, 593.

<sup>112</sup> Awapara, *J. Biol. Chem.*, 1949, **178**, 113; *Arch. Biochem.*, 1948, **19**, 172.

<sup>113</sup> Roberts and Tishkoff, *Science*, 1949, **109**, 14.

<sup>114</sup> Dent, *Biochem. J.*, 1947, **41**, 240.

<sup>115</sup> Ames and Risley, *Proc. Soc. Exp. Biol., N.Y.*, 1948, **68**, 131. Young and Homburger, *Fed. Proc.*, 1948, **7**, 201.

<sup>116</sup> Raper and Shaw, *Nature*, 1948, **162**, 999. Finlayson and Hamer, *ibid.*, 1949, **163**, 843.

<sup>117</sup> Dent, Stepka and Steward, *ibid.*, 1947, **160**, 682. Stepka, Benson and Calvin, *Science*, 1949, **109**, 385.

<sup>118</sup> Woolley, *J. Biol. Chem.*, 1949, **179**, 593.

<sup>119</sup> Fromageot and de Garilhe, *Biochim. Biophys. Acta*, 1949, **3**, 82.

<sup>120</sup> Hamer and Woodhouse, *Nature*, 1949, **163**, 689.

<sup>121</sup> Proom and Woiwod, *J. Gen. Microbiol.*, 1949, **3**, 319.

<sup>122</sup> Work, *Biochim. Biophys. Acta*, 1949, **3**, 400.

<sup>123</sup> Polson, *ibid.*, 1948, **2**, 575.

<sup>124</sup> Linggood and Woiwod, *Nature*, 1949, **163**, 218.

above. In large-scale work the production of toxic filtrates of enhanced titre has followed the determination of the amino-acid requirements of *Corynebacterium diphtheriae*.<sup>125</sup> The quantitative examination of a bacteriophage showed that its composition was not significantly different from that of its host.<sup>126</sup>

Another field in which the amino-acid composition of a hydrolyzate is proving of value is that of the ergot alkaloids.<sup>127</sup> These are characterized by the presence in all members of *D*-proline, while the individuals have either *L*-leucine, *L*-phenylalanine or *L*-valine in peptide combination with the proline and either pyruvic acid or dimethylpyruvic acid. Even the purest preparations have been found to have a trace or more of amino acids pertaining to some other member of the group, although in less than one molecular proportion (cf. §3 (f)). In addition, examination of the crude as well as purer preparations of the alkaloid enables a good indication to be obtained of the members of the series present.

Amino acids, whose presence has not been confirmed by isolation, have been reported to occur on paper chromatograms of natural products. Potato juice,<sup>127</sup> pea juice,<sup>128</sup> molasses<sup>129</sup> and portal blood<sup>130</sup> are thought to contain  $\gamma$ -aminobutyric acid, the  $\alpha$ -isomer of which has been considered to be present in the urine in Fanconi syndrome,<sup>131</sup> in portal blood,<sup>130</sup> in hydrolyzates of *Escherichia coli*<sup>131</sup> and free in *C. diphtheriae*.<sup>125</sup> Ackerman and Kirby<sup>132</sup> consider that *E. coli* contains pantonine rather than  $\alpha$ -aminobutyric acid and Proom and Woiwod<sup>131</sup> have failed to detect any new amino acids in the hydrolyzates of twelve species of bacteria. This illustrates the principle, enunciated above, that isolation must be the criterion for the actual presence of amino acids detected in minor amounts on paper chromatograms.

An advantage of the method is the ability to confirm the absence of an amino acid from a natural product. One of the earliest applications was to show that norleucine is not present in spinal cord, and that the material long considered to be this amino acid, isolated by Thudichum in 1880, was actually *DL*-leucine.<sup>133</sup> Bence Jones protein has been shown to be devoid of methionine.<sup>103</sup>

(2) PAPER CHROMATOGRAPHY OF PEPTIDES.—Perhaps the most valuable use for paper chromatography in the future will be the detection and identification of peptides. Already important results have been obtained for two classes of peptides, the natural peptides synthesized by bacteria, including antibiotics, and the peptides produced by hydrolysis of natural products. The simple peptides were shown by Consden *et al.* to run on phenol-collidine two-dimensional chromatograms as elongated spots, almost as satisfactorily as do amino acids. Their positions are rather sensitive to slight changes in conditions. The use of butanol-acetic acid mixtures, either in one- or two-dimensions, has proved superior to either collidine or phenol for peptides such as the polymyxins<sup>84 105</sup> or for peptides produced by enzymic degradation of insulin.<sup>68</sup> The identification of peptides depends upon their amino-acid composition as revealed by removal from the paper, followed by hydrolysis and identification of the amino acids produced.<sup>134</sup> Difficulties have frequently been encountered in removing peptides in micro-quantities of water and the method adopted by the author is to soak the paper in larger quantities of water and remove this by evaporation under diminished pressure. It is a safe working rule to assume any unknown spot to be a peptide until shown to be completely resistant to acid hydrolysis. A serious difficulty when working with partial hydrolyzates is the larger number of peptides encountered, but colour is some guide to their separate identity.<sup>68</sup>

An early example of the second class mentioned above was the identification of the order of the amino acids in gramicidin S<sup>134</sup>; four dipeptides and two tripeptides were identified, showing that five amino acids were joined in a cyclic

<sup>125</sup> Linggood and Woiwod, *Brit. J. Expt. Path.*, 1948, **29**, 283.

<sup>126</sup> Polson and Wyckoff, *Science*, 1948, **108**, 501.

<sup>127</sup> Foster, MacDonald and Jones, *J. Pharm. Pharmacol.*, 1949 (in press).

<sup>128</sup> Brown, Cook and Stewart, *Biochem. J.*, 1948, **43**, xx.

<sup>129</sup> Jones (unpublished).

<sup>130</sup> Dent and Schilling, *Biochem. J.*, 1949, **44**, 318.

<sup>131</sup> Polson, *Nature*, 1948, **161**, 351.

<sup>132</sup> Ackerman and Kirby, *J. Biol. Chem.*, 1948, **175**, 483.

<sup>133</sup> Consden, Gordon, Martin, Rosenheim and Synge, *Biochem. J.*, 1945, **39**, 251.

<sup>134</sup> Consden, Gordon, Martin and Synge, *ibid.*, 1947, **41**, 596.

peptide structure. More complex structures such as insulin, trypsinogen, the gramicidins and many others are now under investigation in a number of laboratories.

The application to the antibiotic field was the first application to the higher peptides. It was found that admittedly impure preparations of polymyxin A (Aerosporin) gave single spots when chromatographed in butanol-acetic acid mixture, although stationary in butanol.<sup>84</sup> Material obtained from different strains of the organism could be distinguished either by the different positions taken up on the paper, or by differences in their amino acid composition, and this formed the basis of their classification.<sup>186 108</sup> The polymyxins were recognized by the ninhydrin reaction or by fluorescence. The purest available specimens of different members of the series appear to be composite, e.g., using Whatman 4 paper and butanol-15 % acetic acid, polymyxins A and C, but not B and E, appear to be composite, while in butanol-25 % acetic acid, the latter are composite while the first two give single spots.<sup>186</sup> Whatman 7 paper gives better separations of the latter.

Another important class of natural polypeptides contains those synthesized by bacteria in their normal course of growth. Proom and Woiwod<sup>121</sup> have shown that when grown on a standard casein hydrolyzate medium many species of bacteria produce material appearing on the chromatogram as strong ninhydrin-positive spots in positions distinguishable from the standard pattern of amino acids obtained for the basal medium. A number of them have yet to be proved to be polypeptide, but certain of them yield amino acids on hydrolysis. Their appearance in the medium is always associated with the disappearance of one or more amino acids. The presence of polypeptides in the medium may be group-, genus-, or species-specific and may provide data for a classification associated with their nitrogen metabolism. The interior of the bacterium does not appear to contain polypeptide.

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<sup>186</sup> Jones, *ibid.*, 1948, **43**, xxvi.

<sup>108</sup> Jones and Wilkinson (unpublished).

## SEPARATION OF BASES AND AMINO ACIDS BY DISPLACEMENT CHROMATOGRAPHY ON ION-EXCHANGE COLUMNS

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Displacement chromatography possesses special advantages for preparative procedures since the maximum use is made of the adsorptive capacity of the column. In the separation of amino-acid mixtures, columns packed with synthetic resinous ion exchangers may be used and since these are capable of adsorbing large amounts of electrolyte, the throughput of a single column of moderate size may be very large indeed.

Protein hydrolysates may be fractionated by first removing the aromatic amino acids (tyrosine and phenylalanine) by adsorption on charcoal and then displacing the remaining mixture from a column packed with a cation-exchange resin by means of a strong base such as ammonia or sodium hydroxide. A single passage serves to break down the mixture into seven or eight groups of components, each group containing a simple mixture capable of further separation either by conventional methods or by variations of the initial procedure.

The object of the work which forms the subject of this paper was primarily to develop a preparative scale procedure for the isolation of bases and amino acids from biological extracts of various kinds and from the hydrolysis products of proteins. The use of ion-exchange separations for the quantitative analysis of such mixtures has not yet been examined in any detail.

The principle of displacement development was introduced by Tiselius<sup>1</sup> in 1943 and has been applied with success to chromatographic separations on adsorbents such as charcoal and alumina. The work reviewed here, which has been published (in part) in a series of papers<sup>2,3,4</sup> was based very largely on the theoretical background developed by Tiselius and Claesson.<sup>5</sup>

The displacement method applied to ion-exchange columns is of particular value for preparative work since very large amounts of material may be placed on the column. The usual procedure is to pass into the filtration tube sufficient of the mixture of organic electrolytes to saturate about

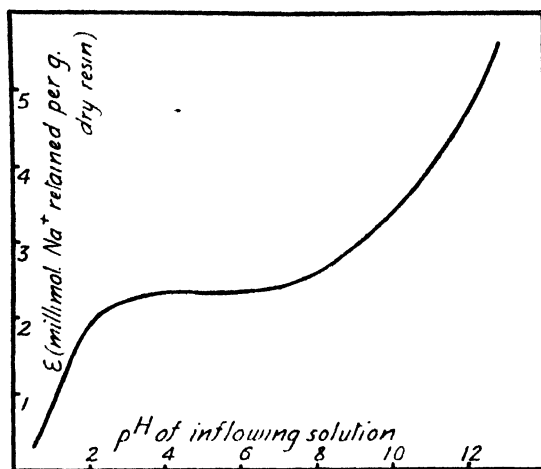


FIG. 1.—Zeo-Karb 215; adsorption of  $\text{Na}^+$  from a range of buffers containing  $\text{NaOH}$  (0.053 N) adjusted to pH 12.1–pH 1.1 by the addition of acids.

one half of the column. Where a cation-exchange resin is used the displacement developer is a solution of a base that has a higher affinity for the column material than any of the components of the mixture. This is introduced onto the top of the filtration tube and, as it displaces the components down the column, they separate into discrete bands, and arrange themselves in order so that each component is displaced by the next strongest base.

**1. Properties of the Resin in Relation to the Displacement Chromatogram.**—(a) The ion-exchange resin that has so far been adopted for most of the experiments with amino acids is the Permutit product Zeo-Karb 215. The properties of this resin may be described by reference to a titration curve (Fig. 1). In carrying out experiments designed to assess the behaviour of resins we have usually preferred to rely on direct observation of columns rather than on equilibrium studies. Thus the points given in Fig. 1 were determined by

<sup>1</sup> Tiselius, *Ark. Kemi, Min. Geol. A*, 1943, **16**, No. 18.

<sup>2</sup> Partridge and Westall, *Biochem. J.*, 1949, **44**, 418.

<sup>3</sup> Partridge and Brimley, *Biochem. J.*, 1949, **44**, 513.

<sup>4</sup> Partridge, *Biochem. J.*, 1949, **44**, 521.

<sup>5</sup> Claesson, *Ark. Kemi, Min. Geol. A*, 1946, **23**, No. 1.



measuring the breakthrough volume of various buffers containing a fixed concentration of sodium hydroxide adjusted to various pH values between 1.1 and 12.1 by the addition of various acids. The curve is obviously a titration curve, but differs from the usual form in that the concentration of sodium ion is constant. The portion of the curve between pH 1 and pH 7 is due to the titration of the sulphonic acid radicles of the resin, and that between pH 7 and pH 12 is due to the titration of phenolic hydroxyl groups. Thus between pH 1 and pH 7 the resin reacts as though it were mono-functional, but at higher pH values a range of weakly acidic groups is titrated. The curve suggests that some of the phenolic groups are more strongly acid than would be expected from the published pK value of phenol, and this may be due to modification of the phenolic groups by oxidation.

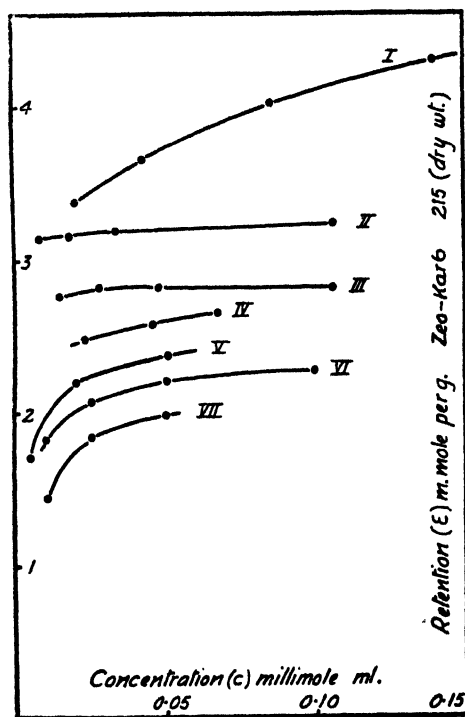


FIG. 2.—Retention of a number of bases and amino acids on Zeo-Karb 215 (80–100 mesh). The experiments were carried out with a column 8.5 mm. diam. containing 1.44 g. resin (dry wt.). I, ammonia; II, lysine; III, histidine; IV, creatinine; V, glycine; VI, glutamic acid; VII, aspartic acid.

(b) Fig. 2 shows a number of adsorption isotherms obtained with various bases and amino acids, again by measurement of the breakthrough volume in column experiments. The adsorption of the various components is clearly determined to a great extent by the pH of the solution applied to the column, and the amount of each component adsorbed follows, more or less, what would be expected from the titration curve. All the natural amino acids are adsorbed from pure solution by Zeo-Karb 215, including aspartic and glutamic acids, the isotherms of which lie below that of glycine.

By use of the graphical method of Tiselius and Claesson<sup>5</sup> the curves given in Fig. 2 may be used for the determination of the concentration at which the components in a displacement experiment leave the column. This method has been found to give good results with mixtures of amino acids displaced with barium hydroxide or ammonia.

(c) Fig. 3 shows the results of one of the earliest experiments in which the displacement technique was applied to the problem of separating organic bases on a column packed with a cation-exchange resin (Bendall, Partridge and Westall).<sup>6</sup> A solution containing glycine and creatinine was applied to the column and was displaced with ammonia. The shape of the fronts and the position of the bands were determined by continuous measurement of electrical conductivity and pH of the solution flowing from the column. The results obtained by this method were afterwards checked by estimation of the content of glycine and creatinine in fractions cut from the effluent. The results obtained in this experiment, which was carried out with a column 10 cm. high, were quite typical, and similar results are obtained in experiments with a larger number of components, although in this case longer columns are necessary.

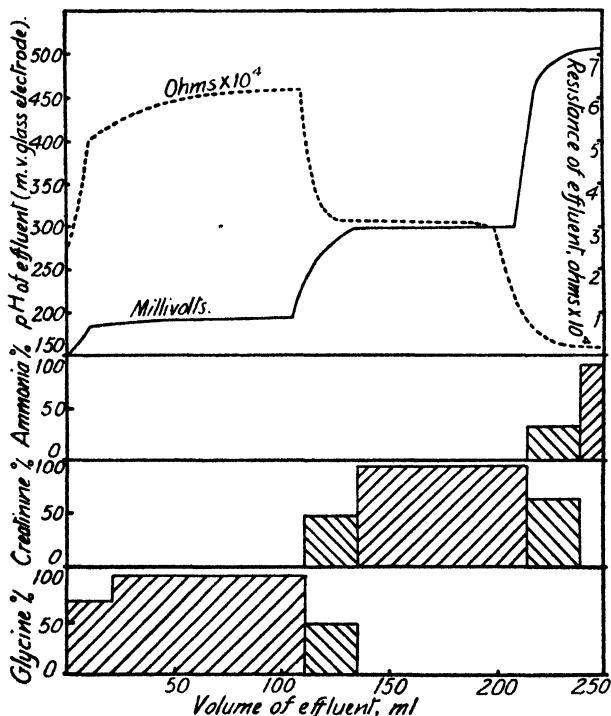


FIG. 3.—Separation of glycine and creatinine on a column of Zeo-Karb 215 (80–100 mesh). The upper part of the figure shows the changes in conductivity and pH of the effluent and the lower part the results of chemical analysis of successive fractions. The analytical results are expressed as percentage of the expected concentration of the component as calculated from adsorption isotherms determined in independent experiments.

--- ohms  $\times 10^4$ , — millivolts.

(d) Obviously, in displacement experiments the yield of pure component obtained depends upon the sharpness of the boundaries of the bands. The effect of various factors on the widths of the boundaries has therefore been studied in some detail and Fig. 4 shows the effect on the width of the boundary of ammonia fronts due to changes in the particle size of the resin and the rate of flow. The co-ordinates of this diagram need some explanation: Fig. 5 is a diagrammatic representation of the concentration changes in the effluent from a column as the solution of a base is passed through it at uniform concentration. The effluent at first consists of pure solvent and the volume of

<sup>6</sup> Bendall, Partridge and Westall, *Nature*, 1947, **160**, 374.

effluent measured to the point of first breakthrough of solute is  $V_0$ ;  $V_{90}$  is the volume of liquid measured to the point at which the concentration of the effluent solution reaches 90 % of that flowing into the column.  $V_e$  is the retention volume of the column and is estimated by fixing the position of the line DB such that the area ABC equals the area ADE. Thus the weight of solute adsorbed by the resin is given by  $CV_e$ , where  $C$  is the concentration of the inflowing solution. The values for  $V_0$ ,  $V_{90}$  and  $V_e$  must be corrected for a small volume  $V_i$  representing the volume of water contained in the apparatus before the experiment begins.

It follows from proportionality that if the length of the column is  $L$  cm. and the width of the boundary of the band due to the solute is  $\lambda_{90}$ , then

$$\frac{\lambda_{90}}{L} = \frac{V_{90} - V_0}{V_e} \quad (1)$$

The volumes in the right-hand side of this equation are readily measured, and thus  $\lambda_{90}$  may be evaluated. It should be pointed out, however, that boundaries

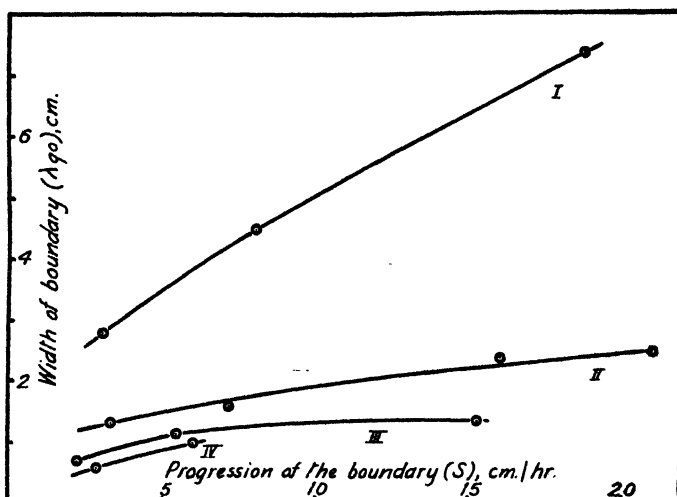


FIG. 4.—Showing the effect on the width of the boundary of an ammonia front due to changes in the particle size of the resin and the rate of progression of the boundary down the column. Zeo-Karb 215: I, 20–40 mesh/in.; II, 40–60 mesh/in.; III, 80–100 mesh/in.; IV, 100–120 mesh/in.

in experimental columns are frequently more or less oblique and this obscures the physical meaning of the boundary width when the measured values of  $\lambda_{90}$  are of the same order as the distortion produced by irregularities in packing. Boundary widths greater than 3–4 cm. may be measured with a fair accuracy, and in this case  $\lambda_{90}$  may be correlated directly with the rate of the exchange reaction taking place on the resin. If the downward rate of progression of the boundary is  $S$  cm./hr. and the boundary width is  $\lambda$  cm. then the time (in hours) required for completion of the exchange reaction on any particle of resin is  $\lambda/S$ .

Inspection of Fig. 4 shows that the boundary given by dilute ammonia solution is very roughly proportional to the rate of progression  $S$  except at low values of  $\lambda_{90}$ . The boundary width varies with a power of the particle diameter and, from the data, there appears to be no advantage (under the conditions envisaged) in using finer resin than 80–100 mesh/in. In general, resin of this particle size has been used for fractionation experiments with small columns, but in experiments with long columns, where the resistance of the resin bed to the flow of solvent is an important consideration, we have usually used 40–60 mesh/in. resin. The rates of flow in these experiments have been adjusted to correspond with rates of progression of the boundaries of 10–15 cm./hr.

The values of  $\lambda_{00}$  given in Fig. 4 refer to the boundary of the ammonia front when the ammonium ion is displacing the hydrogen ion from the acid form of the resin. However,  $\lambda_{00}$  may also be measured for the case where one base displaces another from the column. Thus  $\lambda_{00}$  may be measured for the glycine-ammonia boundary, by saturating the column with glycine and displacing it with ammonia. Generally, such boundaries are a little wider than those obtained with the hydrogen form of the resin, but all such boundaries are of the same order of width. Thus when a series of components are displaced down a long column, and sufficient time and column length have been allowed to ensure that separation is complete in each case, the widths of the boundaries between the various components are of the same order, and in most experiments with *weak bases* the values vary from 0.5 cm. to 3 cm. It should be made clear, however, that the symbol  $\lambda_{00}$  refers specifically to the condition of flowing equilibrium after separation is complete, and that the conception serves little useful purpose where two components of similar affinity for the resin undergo a progressively increasing separation as they pass down the column.

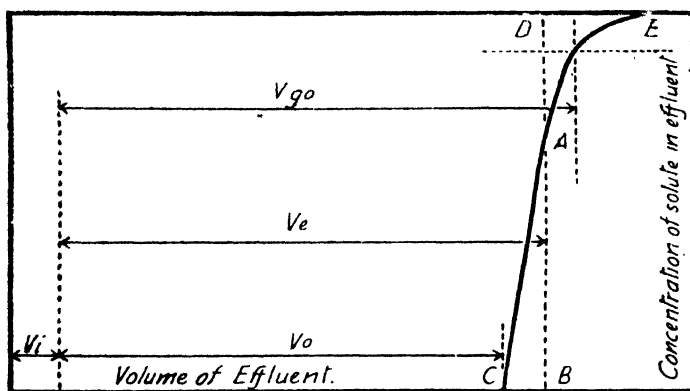


FIG. 5.—Concentration changes in the effluent from a column packed with a cation-exchanger during the passage of a solution of a base (diagrammatic). An explanation of the symbols appears in the text.

(c) With Zeo-Karb 215 it is found that where strong bases are used the values for  $\lambda_{00}$  may become very large indeed, and for that reason it is impracticable to use this resin for bases stronger than ammonia. The reason for this effect of strong bases is clear from the experiment illustrated in Fig. 6. The curves show the shape of the boundaries obtained by passing through a column a series of buffers containing equal concentrations of sodium ion, but adjusted to different pH values by use of a range of weak acids. It will be seen that the boundaries are very sharp from pH 1-7 but get progressively wider as the pH rises from 7-12. At high pH values the boundaries are obviously quite useless for the purpose of separation experiments. If this diagram is compared with the titration curve (Fig. 1), it will be seen that the boundaries become more diffuse as the phenolic hydroxyl groups are titrated. Thus in alkaline solution the resin behaves as if it were a mixture of two ion exchangers, one reacting very rapidly and the other very slowly. This feature of the resin is a serious disadvantage since it limits the range of pH over which practical operations are possible.

**2. Separation of the Amino Acids of a Protein Hydrolyzate.**—When the number of components in the mixture is small the various boundaries flowing from the column may be located by following changes in electrical conductivity and pH by means of cells placed in the path of the effluent, but with a complex mixture such as a protein hydrolyzate this method is insufficiently sensitive and it is necessary to resort to other means of following the changes in composition of the effluent. In recent work the method adopted has been

to divide the effluent arbitrarily into a large number of equal fractions, and to carry out on each fraction a qualitative analysis by means of the filter-paper technique described by Consden, Gordon and Martin.<sup>7</sup>

In a typical experiment the HCl-hydrolysis product of commercial egg albumen (64 g.) was fractionated on a column of Zeo-Karb 215. Since the amino acids tyrosine and phenylalanine behave irregularly on the column, these were first removed by adsorption on prepared charcoal. A partial recovery of tyrosine and phenylalanine was obtained by eluting the charcoal with aqueous phenol-acetic acid.<sup>8</sup> Treatment with charcoal had a further advantage since soluble humin was removed and the charcoal filtrate was clear and colourless.

The column of Zeo-Karb 215 was in two sections, each 82 cm. high and each containing 200 g. of the resin. The mixture of amino-acid hydrochlorides (containing a small excess of hydrochloric acid) was applied to the column, and the mixed band so obtained was developed by displacement with 0.15 N  $\text{NH}_3$ ; the effluent solution was collected by means of an automatic arrangement in 64 fractions, each of 90 ml. The flowing chromatogram was analyzed by paper

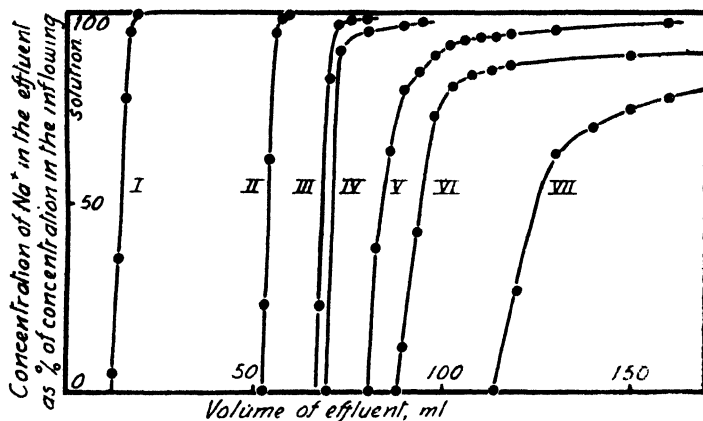


FIG. 6.—Volume-concentration curves showing the shape of the fronts obtained on Zeo-Karb 215 (80–100 mesh/in.) with buffer solutions containing  $\text{Na}^+$ , 0.53 N and various anions. I,  $\text{NaCl} + \text{HCl}$  (pH 1.1); II,  $\text{NaCl} + \text{HCl}$  (pH 2.1); III,  $\text{NaAc} + \text{HAc}$  (pH 4.7); IV,  $\text{NaH}_2\text{PO}_4 + \text{NaOH}$  (pH 6.9); V, boric acid +  $\text{NaOH}$  (pH 9.35); VI, boric acid +  $\text{NaOH}$  (pH 11); VII,  $\text{NaOH}$  (pH 12.5).

chromatography and the result showed the presence of seven discrete bands: I, aspartic acid; II, glutamic acid, serine and threonine; III, glycine and alanine; IV, valine and proline; V, leucine, *isoleucine*, methionine and cystine; VI, histidine and glucosamine; VII, lysine. Arginine has a greater affinity for the resin than ammonia and thus it remained on the column.

The results of the separation are illustrated by Fig. 7, which is a diagrammatic representation of the paper chromatograms. It will be observed that the overlapping portions between the various mixed bands are all of similar width. This indicates that sufficient column length was allowed to complete the separation between the mixed bands; there is indication of partial separation of some of the components within the mixed bands but, obviously, a column of great length would be required to effect the complete separation of all the components of a protein hydrolyzate in a single passage.

However, displacement chromatography may be used to effect a further separation of the components of the individual bands by modifying the experimental conditions. Some of the possible modifications that have been investigated are as follows: (i) the use of a water-soluble organic solvent such as ethanol or acetone in order to modify the ionization of the carboxylic acid groups

<sup>7</sup> Consden, Gordon and Martin, *Biochem. J.*, 1944, **38**, 224.

<sup>8</sup> Schramm and Primosigh, *Ber.*, 1943, **76**, 373.

in the amino acids.<sup>4</sup> (ii) The choice of a solvent mixture having a water-solvent partition in favour of the amino acid it is desired to separate.<sup>4</sup> (iii) The choice of a second ion-exchanger with different properties from that effecting the first separation (e.g., the use of an anion-exchange column to follow a separation carried out on a cation-exchange column).<sup>4</sup> (iv) The properties of one or more of the components may be modified by chemical means.<sup>4</sup>

The actual isolation of the amino acids in a crystalline form is a very simple matter since they appear in the effluent solution in an isoelectric condition, substantially free from salts. With aspartic acid, this appeared (in the experiment described above) at a concentration rather in excess of its solubility, and a heavy crop of crystals formed in the receivers soon after they were filled. The other amino acids and amino-acid mixtures were recovered by evaporating the solution to small bulk and even without recrystallization, they were in a

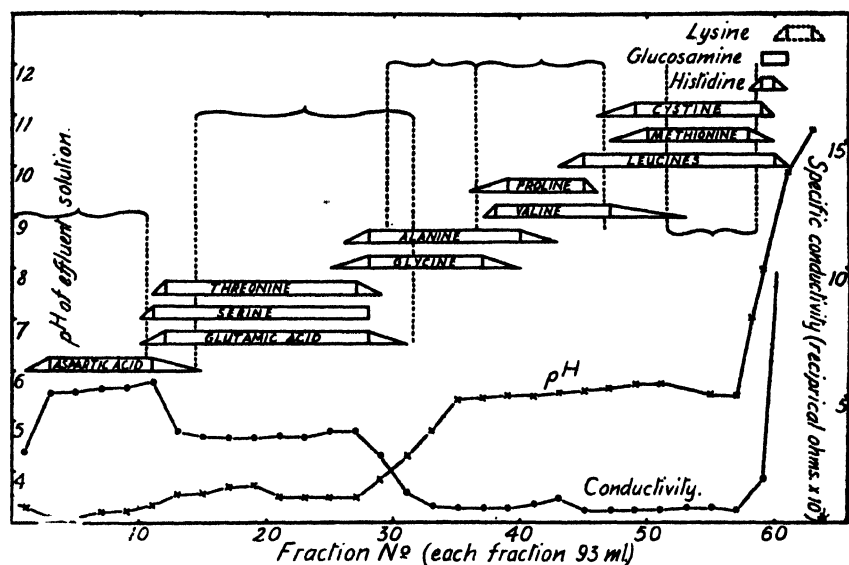


FIG. 7.—Fractionation of a protein hydrolyzate. Analysis of the effluent obtained from a column of Zeo-Karb 215 by displacing the mixture of amino acids with dilute ammonia solution. The upper portion of the figure shows an analysis carried out by means of filter-paper chromatography, while the lower portion shows the corresponding variations in pH and conductivity. The brackets include fractions collected together for treatment on secondary columns.

—●— conductivity, — × —, pH.

substantially pure condition. In all, 46 % of the original protein dry weight was recovered as crystalline amino acids in this experiment, either as the pure substances or as simple mixtures, but it is obvious that this recovery could be very considerably increased by use of longer columns and by returning the mixed fractions forming the boundaries of the bands for refractionation in subsequent experiments.

At the inception of these experiments it was not expected that separations would take place among the homologous mono-carboxylic mono-amino acids since they are alike in acidic and basic dissociation. It has been pointed out<sup>4</sup> that when the displacement developer is a free base, the order of displacement reflects the ability of the stronger base to control the pH of the aqueous phase and thus to depress the cationic form of the weaker bases. However, Davies<sup>4</sup>

<sup>4</sup> Davies, *Biochem. J.*, 1949, **45**, 38.



in order to retain the more basic components. The polystyrene column would then be displaced with sodium hydroxide solution and the Zeo-Karb column by means of ammonia.

With a system such as a protein hydrolyzate, once the primary separation has been carried out, the resulting fractions contain a few components only and are easily dealt with, either by the conventional methods of fractional crystallization or solvent extraction or by further column procedures. Where further chromatography is indicated it is of interest that this may frequently be carried out by use of the same resin adopted for the primary separation, merely by altering the environment of the ions in the solution phase. Thus displacement chromatography offers a considerable advantage over the process of distillation, which it otherwise resembles, since environmental factors may be varied at will, and the best use may therefore be made of differences in the chemical structure of the components in order to separate them. The procedure has been tested to some extent by carrying out the fractionation of the products of hydrolysis of a protein, and in the initial work with a small column, some 45 % of the original weight of protein was recovered either as pure amino acids or as simple mixtures which contained no contaminating impurities and were readily crystallized.

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Cambridge.*

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## CHROMATOGRAPHY OF STEROIDS AND OTHER COLOURLESS SUBSTANCES BY THE METHOD OF FRACTIONAL ELUTION

BY T. REICHSTEIN AND C. W. SHOPPEE

*Received 14th July, 1949*

The method for the separation of coloured substances called chromatography was developed and so named by Tswett (1906), but attracted no attention for a long time until revived by R. Kuhn and E. Lederer.

Many methods have been suggested for the separation of substances which are colourless and give no visible reaction upon irradiation with ultra-violet light. This paper deals only with the technique of fractional elution as broadly applied by us respectively since 1933 and 1939, using almost exclusively  $Al_2O_3$  as adsorbent. The technique is quickly acquired and gives reliable results to those not deterred by its laboriousness.

The mixture of substances is adsorbed on  $Al_2O_3$  from a suitable solvent (as high up in the series given below as solubility allows), and then fractionally eluted by consecutive treatments with solvents of increasing eluting power. The following sequence of solvents has been proved efficient: pentane, petroleum ether, benzene, ether, chloroform or acetone (possibly ethyl acetate), methanol (only rarely with addition of water or acetic acid). Each fraction is evaporated separately, weighed and, if possible, characterized by melting point or other suitable data.

Chromatography is an indispensable tool in steroid chemistry. In rare cases compounds are altered or destroyed during the procedure. The technique described is especially suitable for crystallizable, colourless compounds. Acids and polyphenols react with and remain firmly attached to  $Al_2O_3$ . For the separation of such compounds use of  $SiO_2$ , active carbon, in some cases acid-washed  $Al_2O_3$ , etc., or partition chromatography is indicated.

The present paper contains some observations on the practical procedure for chromatography by fractional elution (*Durchlaufmethode*) as applied by us since 1933 and 1939 respectively.



The Russian botanist Tswett observed in 1906 that mixtures of coloured substances could readily be separated by filtration of a solution through a column of a suitable absorbent followed by subsequent washing of the column with appropriate solvents. The components were thereby concentrated in clearly defined zones, and could be obtained in a pure state by dissection of the extruded column. Tswett named this procedure chromatography, but it remained unnoticed until R. Kuhn and E. Lederer realized its significance and commenced to make full use of it. Since then chromatography has proved itself to be one of the most important techniques for separation and purification.

It is clear that the principles used by Tswett are also applicable to the separation of colourless substances, but that any separation of the components achieved cannot directly be observed. In individual cases this difficulty may be overcome by irradiation with ultra-violet light and observation of the resulting fluorescence. When this is not possible various other methods have been suggested :

(i) Arbitrary dissection of the column into small sections and examination of the material eluted from the individual sections.

(ii) Painting of a narrow strip along the extruded column with reagents, e.g., potassium permanganate (brush method).

(iii) Addition of, and so simultaneous chromatography with, coloured dyestuffs (indicator method).

(iv) Addition of fluorescent substances to the adsorbent ; quenching of the fluorescent often occurs at the enriched zones.

For the separation of colourless compounds we have invariably preferred to use the method of fractional elution, the technique of which will briefly be described. This method is quite well known, but in our opinion too little employed ; it is relatively time-consuming, but gives good results and is extremely reliable. For separations in the laboratory quantities of 30 g. or less are suitable ; larger quantities require semi large-scale equipment for the distillation of solvents. In regard to small quantities, in theory there is no lower limit, but in practice the separation of quantities less than 10 mg. is infrequent because the compounds obtained are then insufficient for micro-analysis. As adsorbent we have used almost exclusively aluminium oxide ; this is suitable for both neutral and basic substances and also for simple phenols. Acids and polyphenols react chemically and are extremely difficult to remove from the column ; for such substances the use of silica, charcoal, acid-washed aluminium oxide or the employment of partition chromatography may be more suitable.

### Experimental

**Preparation of  $\text{Al}_2\text{O}_3$ .**—Activated and partly standardized  $\text{Al}_2\text{O}_3$  is available commercially. Equivalent and very active preparations are obtained by heating technical pure  $\text{Al}(\text{OH})_3$  for about three hours with stirring at  $380^\circ$ – $400^\circ$  ; such preparations always contain free alkali (or sodium carbonate) which, however, is usually not deleterious, and in general give the best separations. In certain cases these preparations are too active and lead to condensation of ketones or aldehydes, elimination of alcoholic hydroxyl groups, etc., but by homogeneous addition of moisture their activity can be reduced and standardized (e.g., by the use of selected dyestuffs under defined conditions<sup>1</sup>).

**Alkali-free  $\text{Al}_2\text{O}_3$ .**—For sensitive substances (ketones, lactones, readily hydrolyzable esters, etc.) neutralized aluminium oxide is used. Aluminium oxide, prepared in the laboratory by activation at  $380^\circ$ – $400^\circ$  or obtained commercially, is boiled repeatedly with distilled water until the extract is neutral ; the filtered material is then washed with methanol and reactivated at  $160^\circ$ – $200^\circ$

<sup>1</sup> Brockmann and Schodder, *Ber.*, 1941, 74, 73.

(internal temperature) at 10 mm. pressure. Preparations reactivated at 200° are too active for many purposes, and a temperature of 180° is generally sufficient. The product still contains traces of alkali, which are not harmful, and is rather less effective than the alkali-containing oxide in regard to separation.

Neutralization is more rapidly achieved by neutralization of the first aqueous suspension with dilute nitric acid (use of hydrochloric, sulphuric or acetic acid is rather more dangerous) followed by boiling with distilled water, treatment with methanol and reactivation as above. The product contains nitrate ions, which are not always harmless.

**Regeneration.**—Used aluminium oxide is repeatedly extracted, first with boiling methanol and then with boiling water (with addition of some sodium hydroxide if necessary) and subsequently treated as described above. Slight discoloration of the regenerated material is of no importance.

**Solvents.**—The following solvents are most frequently used and in the given serial order of increasing elutary power: pentane, petroleum ether, benzene, ether, chloroform (or acetone), methanol or ethanol (possibly mixed with ethyl acetate-water or ethyl acetate-water-acetic acid). All should be dry and freshly distilled; commercial chloroform is stabilized by addition of 1 % ethanol, which is not removed.

Pure methanol, water, etc., are used but little; these solvents dissolve quantities of aluminium oxide, from which the fractions obtained must be separated.

If the mixture to be separated is soluble in petroleum ether, the column is prepared in petroleum ether; in general, the column is prepared in that solvent which, in the above series, precedes the solvent in which the mixture to be separated is soluble.

**Preparation of the Column.**—A glass tube with a tap (no grease!) or with a fused-in sintered-glass plate (porosity 4) is used (cf. Fig. 1), and for 1 g. of substance 30 g.  $\text{Al}_2\text{O}_3$  is employed. The following sizes of tube are convenient:

Amount $\text{Al}_2\text{O}_3$ (g.)	Internal diam. (mm.)	Usable length (mm.)	Bore of tap (mm.)
1	8	110	3
2	10	130	3
4	13	160	4
8	16	200	4
15	20	250	4
30	25	300	6
60	32	400	6
125	40	500	6
250	50	600	8
500	65	750	8

The tube is selected so that, when the appropriate quantity of aluminium oxide has been introduced, it is less than half-full.

After insertion of cotton-wool, a porcelain filter-plate or disc of wire-gauze, fitting the tube as accurately as possible, is introduced, followed by an accurately cut circle of filter-paper. The tap is then closed, and the tube filled completely with the chosen solvent. When all air-bubbles have been removed, highly purified sand is poured in to give a layer of approx. 1 cm. thickness, which is covered with an accurately cut circle of filter-paper; 30 g.  $\text{Al}_2\text{O}_3$  are now introduced in a fine stream and allowed to settle, a process which may be promoted sometimes by gentle tapping. The tap is opened, the solvent run out and the tube repeatedly refilled with the filtrate until the aluminium oxide ceases to pack together: it should be noted the column must *always* remain covered with solvent. The column is covered with a circle of filter-paper, on which is placed a small wad of cotton-wool to prevent disturbance of the column by swirling. Finally, the solvent is allowed to run out until the aluminium oxide just remains covered, and the column is ready for use. Gentle positive pressure may be used to accelerate the production of the column and the subsequent chromatography, and is readily applied by means of the simple apparatus indicated in Fig. 1.

The procedure using a tube with a sintered-glass plate is similar but simpler. The tube is filled with solvent and the aluminium oxide introduced directly onto the sintered-glass plate; gentle positive pressure is applied as shown in Fig. 1, and solvent allowed to run out until the column remains just covered. The pressure supply is disconnected, the tube refilled with the solvent and the process repeated until the column ceases to settle; the column is covered with a circle of filter-paper on which is placed a small wad of cotton-wool, and the solvent expelled until the column remains just covered.

The prepared column should be free from pores and should show no cracks; suction is dangerous and easily leads to the formation of cracks in the presence of volatile solvents, and positive pressure should always be employed in preference to suction.

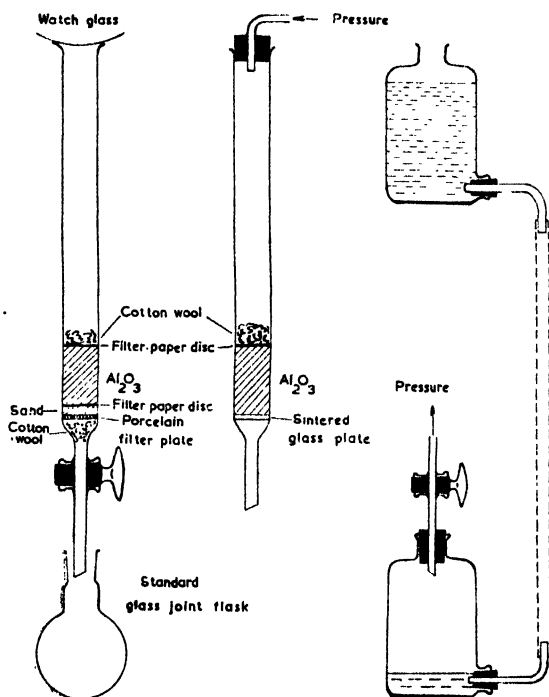


FIG. 1.

**The Actual Chromatography.**—In the case of mixtures soluble in petroleum ether, 1 g. of the substance is dissolved in 100 ml. petroleum ether, the solution allowed to percolate under gravity or gentle positive pressure through the column and the filtrate collected as Fraction 1. Further 100 ml. portions of petroleum ether are allowed to percolate through the column, each fraction being collected separately, evaporated\* and weighed. When the residue obtained by evaporation weighs less than 50 mg., the ascent of the solvent series is commenced by use of benzene-petroleum ether mixtures (starting with 10 %–20 % benzene) for percolation. The proportion of benzene is gradually increased to 50 %, and then directly to 100 %, i.e., pure benzene. Direct passage from one pure solvent to another should never occur because cracks in the column can arise; when passing to ether and especially to methanol, very small proportions of these solvents (1 %, 2 %, 5 %, 10 %) must be used at first.

With a little practice, it is almost always possible to divide a mixture into about 10 or more fractions, of which each should weigh not more than 10 % of

\* Standard-joint flasks and an all-glass apparatus are convenient.

the weight of the original mixture; if some fractions exceed this limit, they must be rechromatographed separately.

For substances insoluble in petroleum ether, a suitable solvent must be chosen and the procedure is somewhat as follows: suppose, for example, the substance is insoluble in petroleum ether and in benzene, but soluble in chloroform. The column is prepared in benzene; 1 g. of the mixture is dissolved in 10 ml. chloroform and the solution diluted with 90 ml. benzene. If a part of the dissolved material is thereby precipitated, the suspension is allowed to settle and the clear solution resulting is poured onto the column. The precipitated material is then redissolved in 10 ml. chloroform, 90 ml. benzene added, and the treatment repeated until all has been dissolved; if this takes too long, the proportion of chloroform can be increased, e.g., 20 ml. chloroform + 80 ml. benzene.

The individual fractions obtained by evaporation of each eluate are examined separately; it is usually possible to decide which are alike on the basis of m.p. crystal form, colour reactions, spectra, etc. Fractions containing the same compound are then united.

It is generally desirable to complete a chromatographic separation in a single day; for rapidity in working, a set of three flasks used in rotation—one for collection of a filtrate, one for evaporation of a filtrate and one with the residue being examined and transferred to a small boiling tube—is helpful. For quantities larger than 1 g., an accurate trial separation of a small sample is to be recommended, because short-cuts often become evident and can be applied to the treatment of the main bulk of material with considerable saving of time.

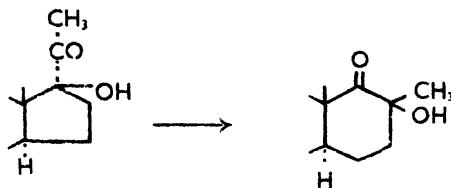
In almost every chromatographic separation, there are observed lightly coloured zones caused by accidental impurities. These should be accurately horizontal; inclined zones or areas exhibiting downward displacements indicate that the column was poorly constructed, and the separation is then generally unsatisfactory.

**Presentation of Results.**—Experimental descriptions of chromatographic separations frequently omit the essential details; the weight and activity of the  $\text{Al}_2\text{O}_3$  employed and possibly the dimensions of the column should be specified, whilst the medium in which the column was constructed and the volume(s) of eluants should be stated. The details of the various fractions may either be tabulated<sup>1</sup> or may be set out in the form of a graph.<sup>2</sup>

### Discussion

By chromatography of coloured substances, it has been possible to achieve results obtainable by no other method. The modification here described is especially suitable for the separation of colourless substances capable of crystallization because by means of melting points the progress of the separation can readily be followed without appreciable wastage of material; in this connection, the use of a micro-m.p. apparatus is particularly desirable. Provided some suitable control is available, the method can equally well be applied to the separation of liquids. Steroids, though colourless, are generally crystalline substances and in this field chromatography has been used with great success: it is in fact almost essential. In a few cases only have difficulties been encountered, e.g.:

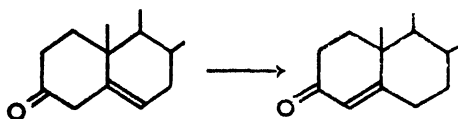
1. 17-Hydroxy-20-ketones, especially those of the 17-isopregnane series, undergo molecular rearrangement to give *D*-homo-ketones. This can largely be avoided by use of neutralized  $\text{Al}_2\text{O}_3$  of low activity and dry solvents, and is completely avoided by use of the 17-acetates.



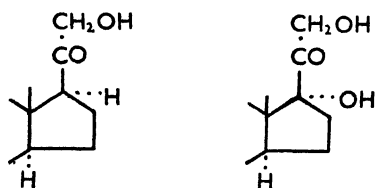
<sup>1</sup> Reichstein and von Euw, *Helv. chim. Acta*, 1941, **24**, 247. Shoppee, *ibid*, 1940, **23**, 925; *J. Chem. Soc.*, 1946, 1138; 1948, 1032; 1949, 1671.

<sup>2</sup> Barton and Miller, *J. Chem. Soc.*, 1949, 337.

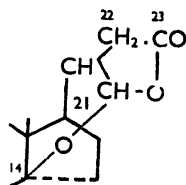
2.  $\Delta^4$ -3-ketones undergo rearrangement to  $\Delta^4$ -3-ketones by use of alkali containing  $\text{Al}_2\text{O}_3$ , but not when neutralized  $\text{Al}_2\text{O}_3$  is employed.



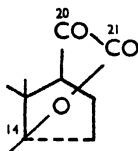
3. Free hydroxy-ketones of the types illustrated often undergo considerable loss. This cannot completely be avoided by use of neutralized  $\text{Al}_2\text{O}_3$ , and it is best to chromatograph the 21-acetates.



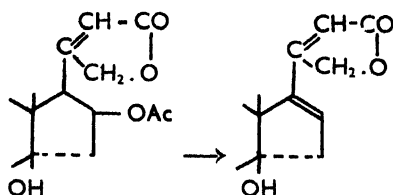
4.  $14\beta$ : 21-oxido-23 $\rightarrow$ 21-lactones ("iso-compounds") derived from heart poisons of the digitalis and strophanthus types undergo large losses probably as the result of facile fission of the 23 $\rightarrow$ 21-lactone ring; such losses are decreased but not completely eliminated by use of neutralized  $\text{Al}_2\text{O}_3$ .



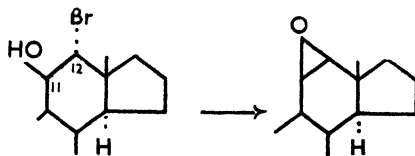
5. 20-Keto-21 $\rightarrow$ 14-lactones suffer very large losses which cannot be avoided even by use of neutralized  $\text{Al}_2\text{O}_3$ .



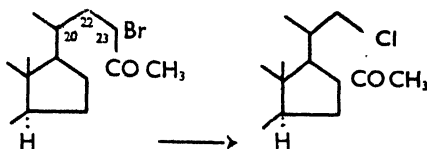
6. 16-Acetoxy derivatives of the type of oleandrigenin or digitoxigenin acetate eliminate one molecule of acetic acid by chromatography on many, but not all, preparations of  $\text{Al}_2\text{O}_3$ .



7. 11:12-Bromhydrins of the type illustrated eliminate hydrogen bromide to give 11 $\beta$ :12 $\beta$ -oxides by filtration over alkaline  $\text{Al}_2\text{O}_3$ , but are unaffected by neutralized  $\text{Al}_2\text{O}_3$ .



8. The 23-bromo-24-ketone shown was found on attempted purification by chromatography on  $\text{Al}_2\text{O}_3$ , which had been neutralized by treatment with dilute hydrochloric acid, despite extensive washing with water after neutralization, to undergo ion exchange to give the corresponding chloro-ketone.



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## PARTITION CHROMATOGRAPHY OF THE TERTIARY AMINE SALTS OF THE PENICILLINS

BY T. LEIGH

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Partition chromatography of the penicillins using a mixture of a tertiary amine and water adsorbed on silica gel as the stationary phase and water-immiscible organic solvents as the mobile phase has been investigated. On applying a solution of the free acids to such a column the penicillins are converted to, and emerge as, the tertiary amine salts. *n*-Heptyl, *n*-amyl, and *p*-hydroxybenzylpenicillins were readily isolated in the pure state from mixtures of these penicillins with  $\Delta^2$ -pentenyl- and benzylpenicillins. Resolution of the last two penicillins was very poor owing to the similarity of the partition coefficients of their amine salts.

The results are compared with those obtained with the phosphate buffer-silica gel-ether system. The microchromatographic assay of Goodall and Levi was used to follow the progress of the separations. Five penicillins of purity > 99.7 % have been isolated and the characteristics of these virtually pure penicillins are appended.

Early attempts to separate mixtures of penicillins by partition chromatography were based upon the method devised by Martin and Synge<sup>1</sup> for the separation of the amino acids. These failed because of the low solubility and pronounced instability of the free penicillin acids in water. The difficulties were overcome by Levi and Terjesen<sup>2</sup> who applied a solution of the penicillin acids in ether to a column composed of silica gel impregnated with

<sup>1</sup> Martin and Synge, *Biochem. J.*, 1941, **35**, 1358.

<sup>2</sup> Levi and Terjesen, *Brit. Pat.* 569,844.

an aqueous phosphate buffer solution and this type of partition chromatography has since been extensively used for the separation of the penicillins produced by the fermentation of *P. notatum* and *P. chrysogenum*.

The major constituents of the mixture of penicillins produced by the submerged fermentation of *P. chrysogenum* Q 176 in a corn steep liquor-lactose-phenylacetic acid medium are benzylpenicillin (penicillin-II or G),  $\Delta^2$ -pentenylpenicillin (I or F), *n*-amylpenicillin (dihydro F) and *n*-heptylpenicillin (K) and it was required to isolate a few grams of each in the pure state to allow the determination of their more important physical and biological properties. Partition chromatography using potassium phosphate buffer and moist ether as the mobile phase afforded four fractions in each of which one penicillin preponderated. *n*-Heptyl- and benzylpenicillin were readily separated from *n*-amyl- and  $\Delta^2$ -pentenylpenicillin and rarely contained more than a few per cent. of the latter, but the resolution of *n*-amyl- and  $\Delta^2$ -pentenylpenicillin was much less efficient and the *n*-amylpenicillin fraction was often heavily contaminated with  $\Delta^2$ -pentenylpenicillin. The sparing solubility of certain amine salts of benzylpenicillin and the relatively much greater solubility of the corresponding salts of *n*-heptylpenicillin in solvents such as acetone and ethyl acetate suggested that the separation of the penicillins might be more easily accomplished by partition chromatography of these salts using water adsorbed on silica gel as the stationary phase and a water-immiscible solvent as the moving phase.

On applying a solution of a tertiary amine salt of the mixed penicillins to such a column the tertiary amine was adsorbed by the silica and the penicillins travelled down as the free acids, separation was poor and some decomposition of the penicillin acids occurred.

Moist silica gel is capable of adsorbing appreciable amounts of organic bases. Thus one part of silica gel impregnated with 70 % of its weight of water adsorbed 0.05 part of N-ethylhexamethyleneimine when treated with a solution of the base in an organic solvent; the base was not removed on washing the silica with neutral organic solvents and a slurry of the treated silica with water had a pH of 7.5-8.0. Chromatogram columns of silica impregnated with water and various amines were prepared and solutions of the penicillins, either as their amine salts or as the free acids, in suitable solvents were charged. On eluting these columns the penicillins passed through in the same order as through a phosphate buffer-silica gel-ether column and emerged as the tertiary amine salts. Because of the low solubility of some of the mixed penicillin salts it was found more convenient to use solutions of the free acids. In the latter case the penicillins are converted to their amine salts at the top of the column by combination with the amine contained on the silica gel. The efficiency of the chromatographic separation did not vary with the method of application. Owing to the instability of the penicillins towards primary and secondary amines only tertiary amines were used. Triethylamine, N-ethylpiperidine and N-ethylhexamethyleneimine were employed for the preparation of the chromatographic columns and ethylene dichloride, chloroform, ethyl acetate and *n*-butanol were tried as eluting agents.

*n*-Heptylpenicillin was very easily separated from the other penicillins by using any combination of the above solvents and bases. For the separation of *n*-amylpenicillin from the remaining penicillins it was necessary to use a solvent in which the penicillin salt had only a sparing solubility compared with its solubility in water and the most efficient system of those examined was that with N-ethylhexamethyleneimine and wet ethyl acetate. For the isolation of those penicillins with the saturated side chains, i.e., *n*-heptyl- and *n*-amylpenicillins, this method proved to be more efficient than partition

chromatography of the mixed acids with phosphate buffer and ether. Comparison of the two types of partition chromatogram showed that for the same ratio of penicillin to weight of silica gel the resolution was sharper and a smaller number of chromatographic separations were required for the isolation of the pure penicillins. Less destruction occurred and the total recovery of penicillin was 10 %–20 % higher than with the phosphate buffer-ether column. The higher recovery is probably associated with the fact that during the separation the penicillin is wholly in the form of the stable tertiary base salt and not as the free acid. The efficiency of the separation was the same whether the starting material was a mixture of the pure penicillins or a crude mixture containing some 40 % of biologically inactive impurities. Improved separation from impurities was also obtained with this type of partition chromatogram. Thus pure crystalline salts of *n*-heptylpenicillin were readily obtained from crude penicillin mixtures but the corresponding fractions from the phosphate buffer-silica-ether columns could not be induced to crystallize owing to heavy contamination with impurities.

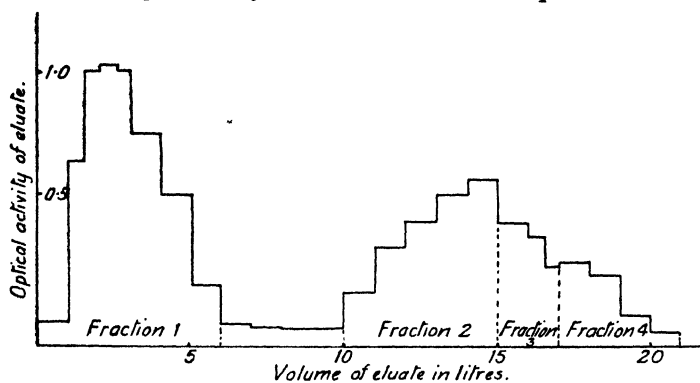


FIG. 1.—Fractionation of mixed penicillins.  
N-ethylhexamethyleneimine salt partition chromatogram.

The tertiary amine–water–organic solvent systems described above failed to separate  $\Delta^2$ -pentenylpenicillin from benzylpenicillin despite the very great difference in solubility of their salts in solvents such as acetone and ethyl acetate. It was presumed that a similar relationship existed between the solubilities of these salts in water and their partition coefficients between water and ethyl acetate at pH 7.0 were found to be approximately equal. Since excellent separation of these two penicillins can be achieved by means of the phosphate buffer-silica-ether column<sup>3</sup> no attempt was made to devise another partition system.

The method has only been used for the separation of the penicillins, but it should be capable of adaptation to the separation of other mixtures of organic acids by the choice of suitable amines and eluting agents and, if necessary, replacement of the stationary phase by other polar solvents and might be of particular value in chromatography of mixtures of acids which, like the penicillins, are only stable in the form of their salts in hydroxylic solvents.

### Experimental

In a typical experiment a stirred suspension of silica gel (500 g.) in ethyl acetate (1500 ml.) was treated with water (350 ml.) over  $\frac{1}{4}$  hr. and N-ethylhexamethyleneimine (25 ml.) was added to the slurry. The suspension was

<sup>3</sup> Boon, Calam, Gudgeon and Levi, *Biochem. J.*, 1948, **43**, 262.



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charged to a chromatogram tube, allowed to settle 1 hr., and the effluent ethyl acetate recycled till the column depth (37 cm.) was constant. A mixture of crystalline sodium penicillins (15 g.) was dissolved in water, the solution was acidified and the acids were transferred to ethyl acetate (100 ml.). This solution was run on to the column which was then developed and eluted with wet ethyl acetate. Twenty 1000 ml. fractions were collected from the moment the ethyl acetate solution was run on to the column. The penicillin content of the fractions was conveniently estimated by measuring the optical activity in a 2 dm. tube using sodium light and the relationship between optical activity and fraction number is shown in Fig. 1.

The first 6 l. of eluate contained almost pure *n*-heptylpenicillin, the next 4 l. had almost zero optical activity and were discarded, the eleventh to the fifteenth litre constituted the *n*-amylpenicillin fraction and the last 5 l. contained

TABLE I  
FRACTIONATION OF MIXED PENICILLINS—N-ETHYLHEXAMETHYLENEIMINE SALT  
PARTITION CHROMATOGRAM

	Percentage of starting material	Percentage composition Penicillin				
		<i>p</i> -hydroxy-benzyl	benzyl-	$\Delta^2$ -pentenyl-	<i>n</i> -amyl-	<i>n</i> -heptyl-
Starting material	—	0.3	4.7	20.0	38.0	37.0
Fraction 1	34	—	0.5	0.5	0.7	98.3
Fraction 2	31	—	0.6	11.0	88.0	0.4
Fraction 3	10	—	6.0	34.0	60.0	—
Fraction 4	13	—	13.0	75.0	12.0	—
Total recovery of penicillin	88					

The above percentages are expressed on a weight basis.

largely  $\Delta^2$ -pentenylpenicillin together with some benzylpenicillin. Four fractions were evaporated to dryness under reduced pressure and the composition of the fractions determined by the microchromatographic method of Goodall and Levi.<sup>4</sup> Details of the composition of the starting material and the four fractions are given in Table I.

The *p*-hydroxybenzylpenicillin was left near the top of the column; it was quite free from contamination by other penicillins. *n*-Heptylpenicillin of purity > 99.9 % was obtained by rechromatographing fraction 1. The overall yield of pure *n*-heptylpenicillin from the starting material, including a recrystallization of the product from the second chromatogram, was 68 %. A further chromatogram with fraction 2 yielded *n*-amylpenicillin of purity 96 %, the remaining 4 % consisting of  $\Delta^2$ -pentenylpenicillin and a final chromatogram yielded *n*-amylpenicillin of purity 99.7 %. After conversion to the sodium salt and recrystallization the overall yield of pure *n*-amylpenicillin from that contained in the starting material was 26 %.

The progress of the fractionation of a similar mixture of penicillins by the phosphate buffer-silica gel-ether method described by Boon *et al.*<sup>3</sup> using amounts of penicillin and silica gel comparable with those used in the N-ethylhexamethyleneimine salt partition chromatogram is indicated in Fig. 2.

<sup>4</sup> Goodall and Levi, *Analyst*, 1947, 72, 277.

In the preparation of this type of column it was found advantageous to 'pour' the column with ethyl acetate instead of the denser ethylene dichloride used by the above authors. Silica gel columns prepared in this fashion showed no tendency to 'crack' after replacement of the first solvent with ether. The eluate was divided into three fractions as indicated in Fig. 2, and a fourth fraction was obtained by washing the silica with water. The penicillin contained in the aqueous extract was transferred to ether and the four ethereal solutions were dried, concentrated under reduced pressure and treated with triethylamine to precipitate the penicillins. Details of the composition of the starting material and the four fractions are given in Table II.

Total recovery of penicillin from these columns averaged 70 % when the separation was performed at room temperature (23° C). The yield was increased to over 80 % when the partition was effected at 5° C. Further purification of the *n*-heptylpenicillin fraction was accomplished by raising the pH of the potassium phosphate buffer to 6.9 and after a second column treatment the amylpenicillin content was reduced to 2 %. A second pH 6.5 phosphate buffer column treatment

TABLE II

FRACTIONATION OF MIXED PENICILLINS—PH 6.5 PHOSPHATE BUFFER—SILICA-ETHER CHROMATOGRAM

	Percentage composition Penicillin				
	<i>p</i> -hydroxy- benzyl-	benzyl-	$\Delta^2$ -pentenyl-	<i>n</i> -amyl-	<i>n</i> -heptyl-
Starting material	2.4	11.0	28.0	28.0	30.6
Fraction 1				14.0	86.0
Fraction 2			21.0	71.0	8.0
Fraction 3		5.0	78.0	17.0	
Fraction 4	2.0	73.0	25.0		

of the *n*-amylpenicillin fraction yielded a main fraction containing 90 % of this penicillin and 10 % of  $\Delta^2$ -pentenylpenicillin and a third column gave *n*-amylpenicillin of 97 % purity. Almost pure benzyl- and  $\Delta^2$ -pentenylpenicillins were obtained on rechromatographing fractions 3 and 4 respectively.

Two criteria were used for assessing the purity of the individual penicillins. Each penicillin was examined for contamination by other penicillins by the microchromatographic assay of Goodall and Levi.<sup>4</sup> This method readily detected the presence of other penicillins when these were present to the extent of more than 0.1 %. The second criterion was the specific rotation and each penicillin was recrystallized till this had a constant value. With the exception of  $\Delta^2$ -pentenylpenicillin the molecular rotations of the penicillins were the same within the limits of experimental error ( $\pm 0.5$  %). In the following descriptions of the properties of the penicillins isolated by the above methods the potencies were determined against *S. aureus* N.R.R.L. 313 and an untyped strain of *B. subtilis* by the cylinder plate method and against *S. aureus* N.C.T.C. 6571 by the turbidimetric method, using pure sodium benzylpenicillin as standard. All melting points are corrected.

***n*-HEPTYLPENICILLIN.**—The anhydrous sodium salt melted with decomposition at 185° and had  $[\alpha]_D^{25}$ , 296° ( $C = 1.0$  % in water) or a molecular rotation of 107,000°. Triethylammonium *n*-heptylpenicillin melted sharply without obvious decomposition at 117°. Microchromatographic assay showed the material to be free from other penicillins and the potencies of the anhydrous sodium salt were: *S. aureus* N.C.T.C. 6571, 2850 u/mg.; *S. aureus* N.R.R.L. 313, 2255 u/mg.;

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*B. subtilis*, 845 u/mg. The corresponding *n*-heptylpenillic acid had m.p. 179° (with decomposition; put in bath at 150°) and  $[\alpha]_D^{25}$ , 489° ( $C = 1\%$  in 0.1 N sodium bicarbonate). Confirmation of the identity of the penicillin was obtained by hydrolysis to *n*-octoic acid in 80 % yield.

***n*-AMYL PENICILLIN.**—The triethylamine, *N*-ethylpiperidine and *N*-ethylhexamethyleneimine salts were extremely difficult to crystallize and the only pure crystalline salt prepared was the sodium salt: m.p., 188° (decomp.);  $[\alpha]_D^{25}$ , 319° ( $C = 0.5\%$  in water); molecular rotation, 107,200°. By microchromatographic assay it contained not less than 99.7 % of *n*-amylpenicillin. Potencies of the anhydrous salt were: *S. aureus* N.C.T.C. 6571, 1680 u/mg.; *S. aureus* N.R.R.L. 313, 1670 u/mg.; *B. subtilis*, 660 u/mg. A preparation of the penillic acid from this material had m.p. 182° and  $[\alpha]_D^{25}$ , 534° ( $C = 1.0\%$  in water). *n*-Caproic acid was obtained in 80 % yield by acid hydrolysis of the penicillin.

**$\Delta^1$ -PENTENYL PENICILLIN.**—This penicillin was isolated by means of the phosphate buffer chromatogram. The sodium salt had m.p. 212° (decomp.);  $[\alpha]_D^{25}$ , 296° ( $C = 1.0\%$  in water) or a molecular rotation of 98,900°. Partition chromatography of the *N*-ethylhexamethyleneimine salt between ethyl acetate and water and subsequent recrystallization of this salt yielded crystals of the same molecular rotation. The *N*-ethylpiperidine and *N*-ethylhexamethyleneimine

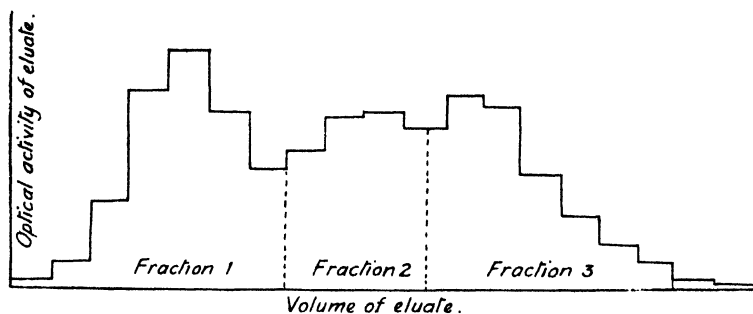


FIG. 2—Fractionation of mixed penicillins.  
pH 6.5 phosphate buffer-silica-ether chromatogram.

salts melted without obvious decomposition at 131° and 115°, respectively. Examination by the microchromatographic assay method indicated a  $\Delta^1$ -pentenylpenicillin content of not less than 99.9 %. The potencies of the anhydrous sodium salt were: *S. aureus* N.C.T.C. 6571, 1930 u/mg.; *B. subtilis*, 970 u/mg. Catalytic hydrogenation by the method of Catch, Cook and Heilbron<sup>5</sup> yielded *n*-amylpenicillin and treatment of the penicillin in aqueous solution with mercuric chloride<sup>6</sup> yielded  $\beta$ -hexenoylaminoacetaldehyde which was identified as the dinitrophenylhydrazone.

**BENZYL PENICILLIN.**—The pure *N*-ethylpiperidine salt had m.p. 163°. Sodium benzylpenicillin melted with decomposition at 225°;  $[\alpha]_D^{25}$ , 303° ( $C = 1.0\%$  in water) or  $[M]_D^{25}$ , 107,900°.

***p*-HYDROXY BENZYL PENICILLIN.**—Unlike the foregoing tertiary amine penicillin salts triethylammonium *p*-hydroxypenicillin melted with effervescence (143°). The sodium salt,  $[\alpha]_D^{25} = 290^\circ$ ,  $[M]_D^{25} = 107,700^\circ$ , had the following potencies:—*S. aureus* N.C.T.C. 6571, 1090 u/mg.; *B. subtilis*, 766 u/mg. The *p*-hydroxybenzylpenicillin content was > 99.9 %.

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<sup>5</sup> Catch, Cook and Heilbron, *Brit. Pat.* 584,852. Improvements in and relating to manufacture of penicillin derivatives.

<sup>6</sup> *The Chemistry of Penicillin* (Oxford).

# BIOLOGICALLY ACTIVE SUBSTANCES IN LIVER EXTRACT

BY E. LESTER SMITH

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Chromatography has been invaluable in the isolation of the anti-pernicious anæmia factor (vitamin B<sub>12</sub>) from liver. Adsorption chromatography of aqueous solutions on charcoal, silica, alumina and aluminium silicate has been employed; also partition chromatography on moist silica, starch and filter-paper, using butanol and other solvents. Propanol (*n*- or *iso*-), though completely miscible with water, gives satisfactory partition chromatograms with damp silica as base. Adsorption on the silica base is superimposed on partition and affects the rate of travel of bands on the chromatograms; nevertheless, partition remains the dominant factor.

Partition chromatography of purified liver extracts on silica, or starch, often reveals two pink zones; the faster one is due to vitamin B<sub>12</sub>, the other to a related substance. On rechromatographing either zone, both reappear; this is presumably due to loose association between the two pigments.

The liver is a remarkable storehouse of vitamins and growth factors, and chromatography has been used in isolating many of them. The fat-soluble vitamins A and D have, for example, been concentrated chromatographically from fish-liver oils. Vitamins of the B group including vitamin B<sub>1</sub> and pteroylglutamic acid have been concentrated from mammalian liver by chromatography of aqueous extracts on ion-exchange resins and other substances. The present paper is concerned with the anti-pernicious anæmia factor, vitamin B<sub>12</sub>, and certain related factors.

Vitamin B<sub>12</sub> was first isolated in crystalline form by Rickes *et al.*<sup>1</sup> by methods that have not yet been disclosed. Within a few weeks it was independently isolated at Glaxo Laboratories<sup>2 3</sup> following progressive purification by adsorption and partition chromatography. Subsequently it has been isolated by Ellis, Petrow and Snook<sup>4</sup> after chromatography on a different adsorbent, namely, aluminium silicate. Details of our methods of isolation, and an account of the properties of this remarkable red cobalt complex of exceptional biological potency, will be published elsewhere.

Certain features of the chromatographic procedures, however, have more general interest and application. A chromatogram is usually made in a tube very much greater in height than in diameter. With a finely divided adsorbent charcoal, however, we have often found it expedient to make these dimensions of the same order, or even to have the diameter much greater than the height; for instance, we have used beds only a few cm. in depth and up to a metre or more in diameter. Our experience with vitamin B<sub>12</sub> has paralleled that with penicillin.<sup>5</sup> In both instances a chromatographic effect can be demonstrated, even with such shallow beds of charcoal; a higher proportion of the active principle is adsorbed from a crude aqueous liquor by passing it through a pre-formed bed than by stirring it with the same amount of charcoal; similarly, less loss of active principle occurs on washing the bed

<sup>1</sup> Rickes, Brink, Koniuszy, Wood and Folkers, *Science*, 1948, **107**, 396.

<sup>2</sup> Lester Smith and Parker, *Biochem. J.*, 1948, **43**, P. 8.

<sup>3</sup> Lester Smith, *Nature*, 1948, **162**, 144.

<sup>4</sup> Ellis, Petrow and Snook, *J. Pharm. Pharmacol.*, 1949, **1**, 287.

<sup>5</sup> Lester Smith, *J. Soc. Chem. Ind.*, 1946, **65**, 308.

with water or with a mild eluting agent (to remove weakly adsorbed impurities) than if it were stirred with the adsorbate. The main advantage of the shallow beds lies in their capacity for rapid treatment of very large volumes of liquor containing valuable but unstable solutes.

Precipitated silica is a weak adsorbent that has some value for further purifying material already containing a few per cent. of vitamin B<sub>12</sub>. We were interested to see whether its adsorptive power could be increased usefully by the technique of Tiselius,<sup>6</sup> namely, the addition to the aqueous solution passed through the column, and to the developing liquid, of an inorganic salt at a concentration just insufficient to salt out the solute. On increasing the concentration of ammonium sulphate up to half-saturation, on a series of columns, a progressive sharpening of the red band was observed, and correspondingly an increase in the capacity of a column of given size. However, persistent impurities (mainly peptides) were not separated much more effectively than they were without the ammonium sulphate.

Partition chromatography on moist silica with butanol and other solvents has been one of the most valuable steps in concentrating vitamin B<sub>12</sub>. By its means a dark brown (but already highly purified) liver extract could readily be resolved into yellow, pink and brown fractions, of which the pink one carried almost the whole of the anti-anæmic activity. Subsequent operations were then simpler because the active fractions could be located by their red colour. Early attempts to set up these partition chromatograms failed because the silica became gelatinous and impervious. The water-logged appearance of the silica suggested that it was abstracting additional water from the *n*-butanol used as mobile phase, which had been saturated with water as was customary. This proved to be so; equilibrium is rapidly established from either direction so that silica containing suitable amounts of water for partition chromatography (i.e., about 60 % of its own weight) is in equilibrium with *n*-butanol containing 11 %–12 % of water by volume (i.e., about two-thirds saturated), which accordingly gives very satisfactory partition chromatograms. Instead of grinding the silica with water, a suitable amount can be conveniently introduced by stirring the dry silica with water-saturated butanol (8 to 10 ml./g. silica) to give a slurry, which can be poured immediately into the tube. The mixture is preferably first degassed by putting it under reduced pressure for about a minute; development of the column can then safely be expedited by suction without air-bubbles appearing.

Propanol (*n*- or *iso*-), though completely miscible with water, behaves in a very similar fashion to butanol, in that liver extracts separate into the same system of coloured bands on silica columns developed with propanol containing 10 %–25 % of water, while the *R* values are also similar. It seems probable that water is redistributed between silica and propanol on contact, as with silica and butanol, so that two liquid phases are created, a water-rich one soaked up by the silica particles and a continuous alcohol-rich one.

Consden, Gordon and Martin<sup>7</sup> have commented that partition chromatograms can be run on filter-paper strips with some water-miscible solvents and have explained this as due to the creation of two liquid phases by a "salting-out" effect of the cellulose. The principle has recently been utilized by Patton and Foreman<sup>8</sup> for the separation of amino acids by partition chromatography on filter-paper with 77 % ethanol.

It is now well recognized that adsorption comes into play, for better or worse, in many of the separations effected by partition chromatography.

<sup>6</sup> Tiselius, *Arkiv. Kemi, Min. Geol. B*, 1948, 26, 1.

<sup>7</sup> Consden, Gordon and Martin, *Biochem. J.*, 1944, 38, 224.

<sup>8</sup> Patton and Foreman, *Science*, 1949, 209, 339.

Martin and Synge,<sup>9</sup> its originators, found it necessary to add a trace of alcohol to the chloroform used to fractionate acetyl amino acids to prevent excessive adsorption. Another early example concerns Sanger's<sup>10</sup> use of fluorodinitrobenzene for end-group analysis of peptides. Attempts to repeat his successful separation of the resulting dinitrophenyl amino acids by partition chromatography on silica failed until a more adsorptive batch of silica was used. We quickly appreciated that this phenomenon was also operating in our fractionations of vitamin B<sub>12</sub>. The pink band of active material travelled out from the almost stationary brown band at the top of the column and then hardly moved any further on continued development, although a fast-running yellow band could easily be developed right out of the column. Different lots of silica varied in their behaviour: with some, it was difficult to separate the narrow brown and pink bands, while others permitted fair separation and gave a more diffuse pink band. Attempts to use starch as a less adsorptive base soon showed the advantages of a mildly adsorptive silica. On starch the pink bands travelled faster, but were so diffuse as to be only just clearly visible, while the capacity of a column of given size was much reduced. Moreover, the purification effected was no better than on silica columns.

Some reviewers, for example, Strain,<sup>11</sup> have called into question the appropriateness of the term partition chromatography (which I was the first to apply to this technique), implying that it cannot properly be distinguished from the original technique of adsorption chromatography. Strain has accordingly proposed a new term, partition adsorbent; this suggestion introduces needless confusion, besides appearing to belittle the spectacular advance made by Martin and Synge, whose method is probably more widely used now than the classical one. Now although adsorption is often involved, the dominant factor is undoubtedly partition; when adsorption occurs, it has the effect of diminishing the concentration of solute in the static aqueous phase below what it would be in the absence of adsorption; the net effect is to diminish the rate of travel of the zone and to sharpen it, especially in front. There is a close analogy with the use of a buffer solution instead of water to moisten the silica in the partition chromatography of acids not very soluble in water, like penicillin,<sup>12</sup> the higher fatty acids<sup>13</sup> and recently dinitrophenyl amino acids.<sup>14</sup> In these instances salt formation instead of adsorption is superimposed on partition, but, unless the acids differ widely in strength, differences in partition coefficient are the main cause of their separation on the chromatograms.

The result is quite different if adsorption alone is relied upon. Two illustrative examples may be cited from the present work. A component that travels quickly on a partition chromatogram must be both readily extractable from water by the solvent and not strongly adsorbed by the supporting solid. A component that travels slowly may on the other hand owe its low *R* value either to a low partition coefficient and little or no adsorption, or to a higher partition coefficient counter-balanced by adsorption. It so happens that vitamin B<sub>12</sub> falls into the second category and the accompanying brown pigments of liver extract into the first; these pigments are nearly insoluble in butanol and not appreciably adsorbed on silica.

A particular sample of this brown material was taken, which had twice been submitted to partition chromatography, each time remaining in the

<sup>9</sup> Martin and Synge, *Biochem. J.*, 1941, **35**, 1358.

<sup>10</sup> Sanger, *Biochem. J.*, 1945, **39**, 507.

<sup>11</sup> Strain, *Anal. Chem.*, 1949, **21**, 75.

<sup>12</sup> Levi, *Biochem. J.*, 1948, **43**, 257.

<sup>13</sup> Moyle, Baldwin and Scarisbrick, *Biochem. J.*, 1948, **43**, 308.

<sup>14</sup> Blackburn, *Nature*, 1949, **163**, 955.

top few mm. of the column despite prolonged development. This material was then submitted to adsorption chromatography on the same solid, silica, from aqueous solution. It now travelled with an  $R$  value of about 1.2, while just above this brown zone appeared a faint pink flush due to a trace of vitamin  $B_{12}$  that had not been completely separated from it by partition chromatography; the position of the vitamin  $B_{12}$  on the column was confirmed by microbiological assay of three adjacent sections. Thus the relative positions of the vitamin  $B_{12}$  and the brown pigments were reversed on changing from partition chromatography (assisted by adsorption) to adsorption chromatography on the same solid with one of the same liquids. Secondly, as mentioned above, propanol containing up to 25 % of water will give excellent partition chromatograms on silica, separating suitable liver extracts into at least three coloured bands. Yet if the water concentration of the developing solvent is increased appreciably above 25 % all these zones are washed quickly and indiscriminately from the column. Thus only when conditions permit the existence of two liquid phases does this system function as a useful chromatogram.

In the course of chromatographic fractionations of liver extract we have had repeated evidence of the separation of two red pigments. Two pink zones have often separated on silica partition chromatograms, especially on rechromatographing a fairly clean fraction. Reproducible separation can, however, be achieved more reliably by using as base starch equilibrated with moist air, with butanol 9/10 saturated with water as mobile phase. The two bands then separate well apart, even though they are rather diffuse, with  $R_F$  values of about 0.5 and 1.2. The faster component corresponds with the crystalline vitamin  $B_{12}$ , the other has not yet been obtained pure.

It is worth noting that this separation fails if the starch is allowed to become too dry, or if development is hastened by applying pressure or suction to the top or bottom of the column respectively. It may be presumed that the rather large molecule of vitamin  $B_{12}$  (molecular weight about 1600 in the hydrated state) requires appreciable time for the successive redistributions between the two phases to approach equilibrium as development proceeds.

The relationship between these two red pigments is not yet established, but it is probably close; solutions of the two pigments of equal colour intensity are also of approximately equal potency, both microbiologically and clinically. A curious feature of their chromatography is the incompleteness of the separation. Thus on a silica partition column two bright pink zones appeared, separated by several cm. of white silica. Yet on eluting and rechromatographing, the major fast zone again splits into fast- and slow-moving components. The fast component had to be rechromatographed a third time to free it completely from the other. These experiments were carried out by Dr. K. Fantes. The incompleteness of the original separation in this and in other similar experiments was confirmed by chromatographic analysis of eluates from the two pink zones on filter-paper strips.<sup>15</sup> The components separated on the paper strips were located and approximately assayed by placing the strips on large plates of suitable nutrient agar inoculated with *L. lactis* Dorner and measuring the maximum diameter of the zones of growth after incubation. It was clear from the results that each pink zone on the silica column contained 20 % or more of the "wrong" red factor. The probable explanation is loose association between the two factors.

The technique described above, of using a sensitive microbiological assay to locate the spots on a paper chromatogram, is interesting in that it probably represents the limit of sensitivity yet achieved. The separation of amounts

<sup>15</sup> Cuthbertson and Lester Smith, *Biochem. J.*, 1949, **44**, P. 5.

of vitamin B<sub>12</sub> and the related factor down to about  $10^{-9}$  g. can readily be demonstrated. Possibly even smaller amounts of "carrier-free" radioactive compounds could be separated and could then be located by radio-autography, but such separations do not appear to have been reported except in presence of relatively large amounts of carrier.

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## HIGH MOLECULAR POLYMERS SEPARATION

BY STIG CLAESSION

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The difficulties associated with chromatographic separation of high molecular substances are discussed. Normally, the rate of adsorption is low requiring an extremely low flow-rate in order to reach equilibrium. The curves obtained by frontal analysis provide information about the composition of high polymer solutions; in special cases, the agreement with other methods of determining molecular weight distribution is good.

It is rather remarkable that very few papers<sup>1-5</sup> dealing with chromatography of high molecular weight substances have been published. If one considers the fact that almost all other types of compounds have been very thoroughly investigated by many different authors, it is even more striking that these of high molecular weight hardly have been studied at all, especially as most of our knowledge about such substances is based upon the use of new and powerful analytical methods.

There are several reasons why we know so little about the chromatography of high molecular substances. It is generally difficult to observe these colourless substances on the column or in the effluent and as they have a continuous distribution of molecular weights no discrete zones will be obtained and it is therefore not profitable to study a limited number of fractions in the effluent. Due to this continuous distribution of material on the column it is also necessary to have very well-packed columns as uneven fronts will cause great errors in the observed distribution of material.

It is also mostly impossible to elute the substance quantitatively from the column getting good separation at the same time. Furthermore very little is known about the very complicated theory for chromatography of substances

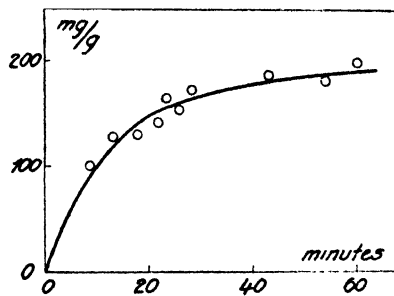


FIG. 1.

<sup>1</sup> Mark and Saito, *Monatsh.*, 1936, **68**, 239.

<sup>2</sup> Levi and Giera, *Gazz. chim. ital.*, 1937, **67**, 719.

<sup>3</sup> Landler, *Compt. rend.*, 1947, **225**, 234.

<sup>4</sup> Claesson and Claesson, *Physic. Rev.*, 1948, **73**, 1221.

<sup>5</sup> Claesson, *Arkiv. Kemi, Min. Geol. A*, 1949, **26**, No. 24.



with continuous distribution of molecular species. However, even if very little quantitative work has been done in this field it seems of interest to discuss the little that is known at present as it may initiate further research.

The most important fairly well-established facts are the following. As can be expected the rate of adsorption is low when the molecular weight is high. The reason for that is certainly the low diffusion constant for such substances. A typical curve <sup>6</sup> showing the low rate of adsorption is shown in Fig. 1, where the amount adsorbed (mg./g.) is given as a function of time for the adsorption of a polyvinyl acetate of molecular weight 22,000 on activated carbon. That causes extra difficulties, as the rate of flow through

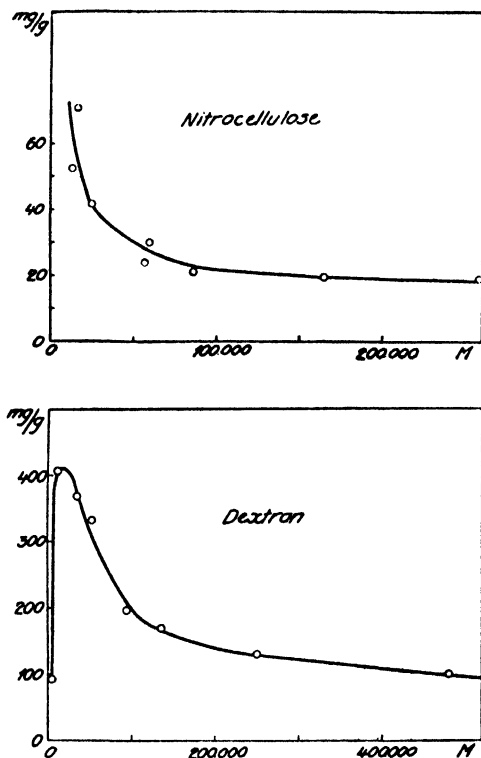


FIG. 2.

the column must be extremely low in order to reach adsorption equilibrium. Otherwise instantaneous equilibrium cannot be assumed in the theoretical treatment and the mathematical difficulties will then be unsurmountable.

The amount adsorbed per gram adsorbent is also generally small, but of course not lower than for many low molecular compounds without polar groups.

For a high polymer homologous series the amount adsorbed usually decreases with increasing molecular weight. That has proved to be the case for many different high polymers.<sup>4 6</sup> That is in marked contrast to the case of low molecular substances where the reverse is true. There must consequently exist a maximum adsorbability for an intermediate molecular

<sup>6</sup> Claesson and Claesson, *Arkiv. Kemi, Min. Geol. A*, 1944, 19, No. 5.

weight (most probably around  $M = 10,000$ ). Two curves <sup>4,7</sup> showing the amount adsorbed (in mg./g. adsorbent) as a function of the molecular weight  $M$  for 1.5 % solutions of nitrocellulose in acetone and dextran in water on activated carbon are given in Fig. 2.

Some experiments have been carried out by the present author in order to investigate the possibilities of using chromatography for a determination of the composition of solutions of high polymers. It is evident that such an investigation has to be arranged with care in order to get useful results in spite of the difficulties mentioned above. In order to avoid the trouble with incomplete elution only frontal analysis was used. The concentration of the solution leaving the filter with adsorbent was followed by measuring

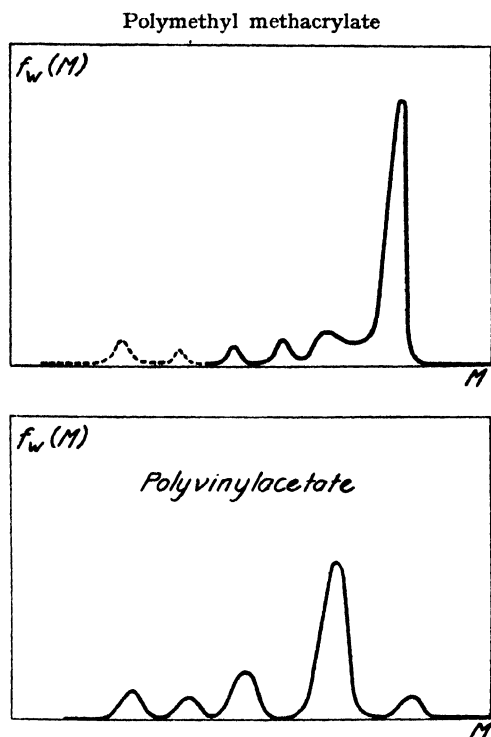


FIG. 3.

the refractive index by a micro-interferometer in the way described by Tiselius and the present author.<sup>8</sup> In order to reach sufficient accuracy (better than  $10^{-6}$  in refractive index) a new improved interferometer was constructed which had a 400 mm. long cuvette. Activated carbon (usually about 1 g. Carboraffin) mixed with three parts by weight of Supercel was used as adsorbent. The system with coupled filters<sup>9</sup> (the solution passes from a filter with a large to a filter with a smaller diameter through a mixing chamber) was used to get good fronts. The rate of flow was kept very low (0.1-0.2 ml./min.) in order to be close to adsorption equilibrium. The most difficult part of the problem is, however, to avoid the use of a complicated

<sup>7</sup> Ingelman and Halling, *Arkiv. Kemi*, 1949, **1**, No. 10.

<sup>8</sup> Tiselius and Claesson, *Arkiv. Kemi, Min. Geol. B*, 1942, **15**, No. 18.

<sup>9</sup> Claesson, *Arkiv. Kemi, Min. Geol. A*, 1947, **24**, No. 16.

theory to compensate for the fact that the different molecular species may displace each other. It has in fact been demonstrated by separate experiments with fractions of high polymers that this effect is fairly large. An attempt to avoid this difficulty was made by using very dilute solutions where such effects are smaller. The diagrams were then extrapolated to zero concentration. This procedure seems to be very useful in general; there is, however, no guarantee that it must give a correct result if it cannot be verified by other means. As very little is known about the adsorption isotherms of high polymer fractions at the present time it was thought that this procedure was the best possible way of getting any results at all. Experiments have therefore been carried out at a series of total concentrations

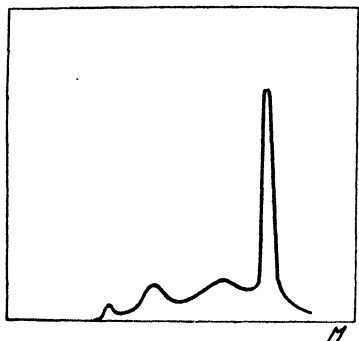


FIG. 4.

down to 0.02 % and extrapolated to zero concentration. It was found that very reproducible results could be obtained in this way which showed very little variation with changes of rate of flow up to 0.3 ml./min. It is quite clear that a diagram from a frontal analysis under such conditions where displacement effects and non-equilibrium are disregarded directly will give information about the composition of the high polymer solution. If a curve of the type given in Fig. 2 is used to correlate molecular weight and retention volume, the result can easily be expressed as the molecular weight distribution function.

Two typical curves obtained in this way are given in Fig. 3 showing the extrapolated curves for a solution of polymethyl methacrylate in acetone and a solution of polyvinyl acetate in acetone. The curves are given as frequency distribution functions.

The great number of marked features on these curves is very remarkable. It is hard to believe that this should be caused only by a very complicated molecular weight distribution. It is more probable that other factors come into play—e.g., different branching of the chain, nature of end-groups, the order of the groups in a copolymerizate and so on. Neither can it be excluded that some components in the diagram are low molecular weight impurities. However, it is obvious that chromatography will give such very detailed information about the composition of high polymer solutions that almost no other analytical method for high polymers can compare with it.

Much still remains to be done in this field of chromatography. One of the most obvious things to try is to use mixed solvents<sup>3,5</sup> (a solvent and a precipitant) to increase the adsorption. This may cause a decrease in selectivity as the larger molecules will be more affected than the smaller ones, but it will be compensated for if such weak adsorbents can be used in this way that quantitative elution is possible. A study of the properties of different adsorbents and particularly the use of partition chromatography will certainly bring forward new results. It will also be very interesting to carry out more experiments with well-fractionated materials of different kinds to get more detailed information about the adsorption properties of such substances.

We do not know to-day if chromatography can give information about molecular weight distribution in general. The agreement with other methods is, however, very good in special cases. Fig. 4 gives the molecular weight

distribution determined<sup>10</sup> from fractionated precipitation and viscosity measurements for the same polymethyl methacrylate as in Fig. 3 and the general shape of these two curves is very similar. Chromatography therefore seems to be an extremely powerful method also for the study of high molecular weight substances, so promising indeed that it may very well happen that this new branch of chromatography in the near future will prove to be one of the most important applications of Tswett's original brilliant idea.

*Fysikalisk-Kemiska Institutionen,  
Uppsala.*

<sup>10</sup> Kinell, *Acta Chem. Scand.*, 1947, **1**, 749.

### GENERAL DISCUSSION

**Dr. G. H. Beaven** (*Thornton Research Centre*)\* said: Work carried out in collaboration with C. N. Thompson and L. F. Foster, which has been submitted elsewhere for publication, has shown that the high affinity of activated silica gel for aromatic hydrocarbons generally can be utilized for the efficient separation of heavy petroleum fractions into total saturate and total aromatic fractions. The technique which we have devised is very similar to that described by Smit<sup>1</sup> and uses isopentane as the initial solvent which conveys the total saturates through the column, followed by, e.g., ether to desorb the total aromatic fraction. Working on an "analytical" scale with sample weights of ca. 20 g. and a 30 in.  $\times$  1 in. column of silica gel (100–200 mesh), essentially complete recoveries can be obtained from distillates and semi- or completely-refined products, and their total saturates and total aromatics estimated to  $\pm 1\%$  or better. This method has been most used in the spindle-oil range, which contains material in the molecular weight range ca. 200–300, but it is also applicable to petroleum fractions extending from gas oils to heavy lubricating oils.<sup>2,3,4</sup> The adsorption analysis of gasolines has been described by several American workers,<sup>5</sup> while Lawrence and Barby<sup>6</sup> have shown that, using alumina as the adsorbent, the method is also applicable to residual oils containing asphaltic material, so that the entire range of liquid petroleum fractions is susceptible to adsorption analysis.

The practical problem of determining the point at which all the saturates have been eluted is best solved by the empirical method, described by Smit<sup>1</sup> and by Reichstein and Shoppee,<sup>7</sup> of continuing elution with a given solvent until the solute content of the eluate becomes negligible. This is conveniently done by using a suitable eluant sampling schedule (or an automatic fraction-cutter) together with some form of rapid solvent-removal device. Refractive index and specific dispersion measurements are also of great value in distinguishing between saturate and aromatic fractions, but it is often difficult to select definite values of these characteristics to indicate the transition point, owing partly to some degree of fractionation within the saturate fraction itself (cf. Smit<sup>1</sup>), and also because of the anomalous refractive indices of some hydrocarbons of mixed structural type. The freedom from aromatics of the total saturate fraction obtained in the manner described above may be confirmed by fluorescence and ultra-violet absorption tests.

<sup>1</sup> This Discussion.

<sup>2</sup> Dinneen *et al.*, *Anal. Chem.*, 1947, **19**, 992.

<sup>3</sup> Lipkin *et al.*, *Anal. Chem.*, 1948, **20**, 130.

<sup>4</sup> Fred and Putscher, *Anal. Chem.*, 1949, **21**, 903.

<sup>5</sup> Cf. Mair *et al.*, *J. Res. Nat. Bur. Stand.*, 1945, **34**, 435; 1944, **32**, 165.

<sup>6</sup> This Discussion.

<sup>7</sup> This Discussion.

\* Present address: M.R.C. Spectrographic Unit, The London Hospital, E.1.

For the separation of the aromatics into several fractions rather than a single bulk fraction, our work has fully confirmed the value of fractional elution recommended by Smit and other contributors to this Discussion. We have used up to five solvents, e.g., isopentane, carbon tetrachloride, ether, acetone and benzene (in this order), but carbon tetrachloride followed only by ether is found to give almost complete elution of the aromatics of light lubricating oils and related products. By fractional elution some separation of the total aromatic fraction

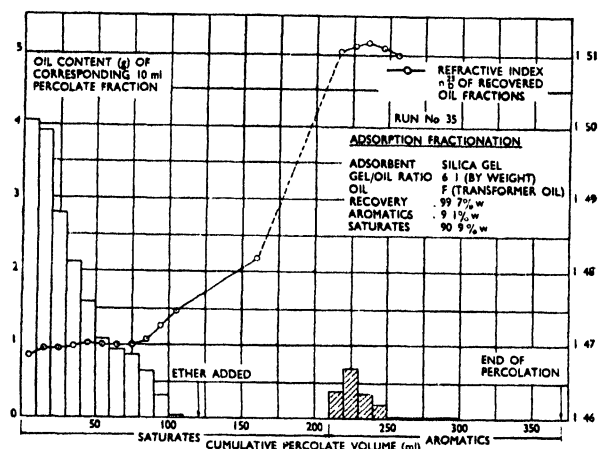


FIG. 1.—Adsorption fractionation.

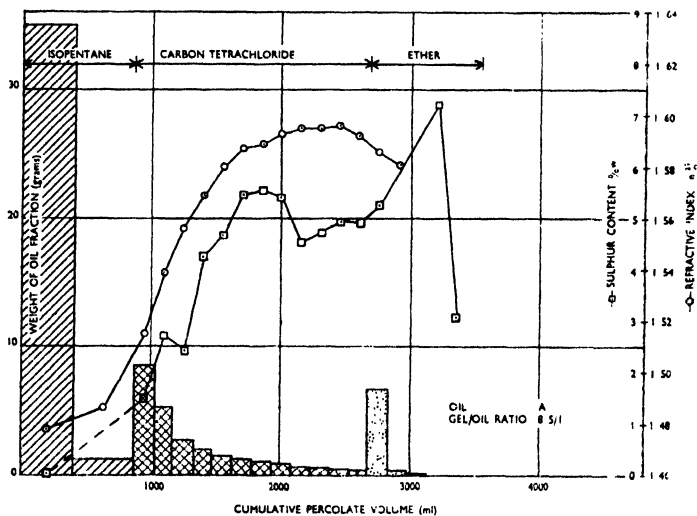


FIG. 2.—Three-solvent desorption analysis.

into different aromatic types can be achieved, as shown by the progressive increases in the refractive indices and specific dispersions of the successive sub-fractions, but the complete separation of, e.g., monocyclic and dicyclic aromatics, which is possible with pure hydrocarbons,<sup>1</sup> has not yet been definitely observed with petroleum products. The problem is made more difficult by the simultaneous segregation of the sulphur compounds, which are mainly associated with the aromatic fractions during adsorption analysis.<sup>6</sup>

Fig. 1 shows a typical separation of a transformer oil into a large total saturate fraction and a small total aromatic fraction; the small transition fraction of intermediate refractive index may be noted. Fig. 2 shows a 3-solvent preparative-scale separation of a crude spindle-oil distillate into a bulk saturate and two aromatic fractions; the variation in sulphur content of the individual aromatic sub-fractions is also indicated. These two examples serve to show the value of adsorption analysis, even in a simple form, for the study of petroleum fractions.

Our work has also confirmed the observation by Smit<sup>1</sup> regarding the photosensitivity of some oil fractions when adsorbed on silica gel. In this connection it may be noted that Ewles<sup>8</sup> has found that water and several polar organic liquids show unexpected fluorescence and phosphorescence characteristics when adsorbed on silica gel and other inorganic solids. These characteristics must presumably be associated with alterations in the light-absorbing properties of the compounds to the extent that in the adsorbed state they can be excited by radiation of much longer wavelength than that required to excite the same compounds in bulk. A similar alteration in light-absorbing properties may be responsible for the photosensitivity of adsorbed petroleum hydrocarbons to visible light.

**Dr. A. S. C. Lawrence** (*Sheffield*) said: Black oils are, as their name suggests, black and include residua from the distillation of petroleum and mixtures of such residua with heavy distillates. The black colour is due to the fraction which we isolate as  $\omega$ . Since the paper was written, ultimate analyses have been made of fraction 57 with the following results:

Fraction		C	H	H/C	O	S	N	Ash
$\alpha$	..	84.3	12.7	1.79	3.0	—	—	—
$\beta$	..	83.3	11.9	1.72	4.5	—	—	0.3
$\gamma$	..	82.9	9.3	1.345	4.05	2.15	—	0.2
$\delta_1$	..	82.5	8.2	1.195	6.2	1.9	—	1.2
$\delta_2$	..	80.7	8.1	1.275	8.9	1.7	—	0.1

These results suggest the rather startling conclusion that the higher fractions of petroleum oil contain no hydrocarbon. The literature is extraordinarily lacking in results of ultimate analyses of all petroleum products. It is considered that the easy elution of fractions  $\alpha$  and  $\beta$  indicates that the oxygen is present in non-polar form and it is obviously not very important from the point of view of fractionation of hydrocarbon types if these very large molecules contain a few  $-C-O-C-$  or  $-C-S-C-$  links in place of  $-C-C-C-$ . However, the higher resinous fractions show signs of polar properties such as their stronger adsorption on alumina, spreading on water, efficiency as water in oil emulsifying agents and flocculation changes with pH. These properties all suggest that some at least of the oxygen is present in polar groups.

The hydrogen/carbon atomic ratios show considerable divergence from the theoretical value of nearly 2 for the paraffin and cycloparaffin types which should be  $\alpha$  and  $\beta$  fractions, and from 1 for the  $\gamma$  aromatic one. The results of the chromatographic fractionation suggest that these divergencies are due to mixing of types in the molecules rather than to any limitations of the method.

It may be noted that the oxygen-containing resins appear to be quite different from the coal tar pitch resins which are also distinguished by high oxygen content. These are easily oxidized by alkaline permanganate to colourless water-soluble products but our  $\gamma$  fraction is not so oxidized.

Although our products are considerably unsaturated, no sign of irreversible adsorption was observed. The literature contains numerous references to the irreversible adsorption of olefins. It is suggested that the preliminary wetting of the alumina by pure solvent, which gives remarkable evolution of heat, so reduces the heat of adsorption of olefins that polymerization does not occur; that is, that irreversible polymerization, when it does occur, is a thermal effect. A number of patents for improving oils by filtration through alumina (bauxite) include wetting of the alumina by water.

<sup>8</sup> Ewles and Farnell, *Proc. Physic. Soc. A*, 1949, **62**, 216.

**Prof. A. Tiselius (Uppsala)** said : In Dr. Cassidy's paper, as well as in several other contributions to this Discussion, the use of active carbon in adsorption chromatography has been exemplified. We have had considerable experience with this adsorbent in my own laboratory, and I would like to point out that, although it has great advantages in its specificity and large adsorption capacity, it has the disadvantage of showing some irreversible adsorption (or slow desorption), particularly with strongly adsorbed substances. In his summing-up of the first section of this Discussion, Dr. R. L. M. Synge mentioned some experiments in which this difficulty had been partially overcome by pretreating the carbon with stearic acid, which blocks the most active patches on the carbon surface. Nevertheless, losses often occur on account of this irreversible adsorption, particularly in elution, and also to a certain extent in displacement analysis.

**Dr. A. J. P. Martin (Medical Research Council Unit, London)** said : Displacement partition columns for acids or bases, the column being loaded with a base or acids, have potentially very large capacities. Probably concentrations in the range of 0.5 to 2 N can be used on the support. A theory for such columns has been given.<sup>9</sup>

The scope of partition chromatography can also be extended to substances with partition coefficients in favour of the non-polar phase by treating silica, precipitated or in the form of kieselguhr (Celite or Hyflo Supercel), with dimethyl dichlorosilane. The silica will then hold the less polar of a given phase pair. With a hydrocarbon on the silica, e.g., medicinal paraffin or octane, and an aqueous solution of acetone or methanol as the mobile phase, it is possible to separate fatty acids differing by two carbon atoms, e.g., lauric, myristic, palmitic and stearic acids.<sup>10</sup>

**Dr. E. Lederer (Paris)** said : Cassidy and Nestler have rightly stressed the difficulties encountered in the purification of carboxylic acids by adsorption chromatography. I wish to report a case of the successful application of chromatography to the purification of high-molecular biologically important carboxylic acids.

TABLE I

SEPARATION OF  $\alpha$ - AND  $\beta$ -MYCOLIC ACIDS (1585 MG. ON 32 G. ALUMINA ACTIVITY II)<sup>11</sup>

Eluting Solvents						Eluate (mg.)	M.p. (° C)
a	150 ml. benzene	..	..	..	..	14.5	—
b, c	300 ml. dry ether	..	..	..	..	4	—
d	150 ml. ether	..	..	..	..	8	—
e	150 ml. ether	0.1 %	acetic acid	..	..	322.5	55-56
f	200 " "	0.1 %	" "	..	..	571	55-56
g	200 " "	0.1 %	" "	..	..	268	55-56
h	200 " "	0.3 %	" "	..	..	194	57-58.5
i	200 " "	0.5 %	" "	..	..	74	—
k	200 " "	1.0 %	" "	..	..	63	62-64
						1519 (96 %)	

Mycolic acid is a branched hydroxy-methoxy acid isolated by Stodola and Anderson<sup>12</sup> from the wax of a human strain of *Mycobacterium tuberculosis* and to which the authors assigned the formula  $C_{88}H_{176}O_4$  (mol. wt. 1298); this is the only acid-fast substance found in the tubercle bacillus. The salts of mycolic acid are soluble in organic solvents, even in petroleum ether, so that it cannot be separated from neutral substances by the usual extraction with aqueous alkali from an organic solvent.

<sup>9</sup> Martin, *Biochem. Soc. Symp.*, No. 3, 1949.

<sup>10</sup> Howard and Martin (unpublished).

<sup>11</sup> Asselineau and Lederer, *Compt. rend.*, 1949, **228**, 1892.

<sup>12</sup> Stodola, Lesuk and Anderson, *J. Biol. Chem.*, 1938, **126**, 505.

In a recent paper, Asselineau and Lederer<sup>13</sup> have described the chromatography of mycolic acid on alumina. Mycolic acid is strongly adsorbed and can be quantitatively eluted by ether containing 5 %–10 % of acetic acid. By this method it was easy to show that different strains of mycobacteria contain free mycolic acid (up to 4 %).

Thus purified, mycolic acid can then be split into two isomeric components  $C_{88}H_{176}O_4$ , which we have called  $\alpha$ - and  $\beta$ -mycolic acids. Table I shows such a separation which uses increasing amounts of acetic acid in ether to elute the two isomers separately. This may be regarded as a displacement development.

We have isolated isomeric forms of mycolic acid from a bovine strain of *Mycobacterium tuberculosis*. The mycolic acid of the B.C.G. strain which was said to have a molecular weight of only 770 can be easily split by chromatography into a neutral part (m.p. 75°) and an acid (m.p. 54°–58°) having a molecular weight of about 1000. (Unpublished results.)

After a second, similar chromatography, fractions *e*, *f*, *g* give  $\alpha$ -mycolic acid, m.p., 55°–56°, and fractions *i*, *k* give  $\beta$ -mycolic acid, m.p. 71°–73°.

**Prof. L. Zechmeister (California)** said: I agree with Prof. Tiselius that the main difficulty in protein chromatography is the recovery of the protein from its adsorbate and the reliable check of its unaltered state. However, this difficulty can be overcome when the protein studied shows well-defined enzymatic (or serological?) properties. Then an unchanged enzyme potency offers sufficient proof in the direction mentioned. In pertinent experiments carried out by Dr. Tóth and others in our laboratory some unexpected variations in the adsorption behaviour of enzymes were observed upon the repetition of the chromatographic experiment. On the basis of the important paper under discussion, it seems possible that these variations were caused by an alteration of the salt content in the initial, crude enzyme extracts during the first adsorption process.

**Dr. R. Consden (Maidenhead)** said: One of the most important applications of paper chromatography is in the separation of peptides from partial hydrolyzates of proteins. The number of peptides may be so large that preliminary separations are essential before paper chromatography can be used to the best advantage. Anion-exchange resins have been used for the separation of acidic from non-acidic material, and ionophoresis has separated the acidic fraction into groups of similar mobilities, each group then being examined by paper chromatography. In this way, some twenty dipeptides of glutamic and aspartic acids have been identified in partial hydrolyzates of wool, and twelve dipeptides of cystine, after converting them to cysteic acid peptides. Only a few lower peptides of neutral or basic amino acids have been identified. These are more difficult to investigate than acidic peptides. The neutral peptide fraction may contain many constituents, which are not easily separated in ionophoresis. Many of them may be too fast running on paper chromatograms with the usual solvents. Basic peptides often do not behave satisfactorily on paper chromatograms and often do not deaminate readily. The problem of adequately separating and analyzing basic and neutral peptides is therefore one which requires further attention.

**Dr. E. Lederer (Paris)** said: Dr. Jones mentions his attempts to isolate  $\gamma$ -aminobutyric acid from molasses; Dr. Dent (London) has told me that he also has difficulties in isolating this substance from potato juice. I wish to report a method of isolating  $\omega$ -amino acids which may be of interest in this respect (to be published with Pavlov).

Some years ago, I have shown with Tchen<sup>14</sup> and then with Jutisz<sup>15</sup> that  $\beta$ -alanine is adsorbed on acid alumina in presence of 10 % formol, just as are glycine, serine and threonine.<sup>16</sup> We had explained the preferential adsorption of glycine by steric considerations and had written "Si vraiment l'effet du formol sur l'acidité du glycocolle est due à la présence d'un  $-NH_2$  lié à un carbone primaire,

<sup>13</sup> Asselineau and Lederer, *Bull. Soc. Chim. biol.*, 1949, **31**, 492.

<sup>14</sup> Lederer and Tchen, *Biochim. Biophys. Acta*, 1947, **1**, 35.

<sup>15</sup> Jutisz and Lederer, *Biologie et Médecine*, No. 5 (*Symposium sur les protéines*), p. 239.

<sup>16</sup> See Schramm and Primosigh, *Ber.*, 1943, **76**, 373.



nous devons trouver le même phénomène chez tous les acides aminés en  $\omega$ ." The first example was  $\beta$ -alanine and we have now extended these experiments to  $\gamma$ -aminobutyric,  $\delta$ -aminovaleric and  $\epsilon$ -aminocaproic acids. Adsorption of these  $\omega$ -amino acids on acid alumina, in presence of 10 % formol, is very dependent on pH. On using columns washed with water until the effluent has a pH of 4.0 ( $\pm 0.1$ ), up to 95 % of the above-mentioned amino acids are adsorbed on the column and can be quantitatively eluted by hot water which dissociates the formol-amino acid complex.

Dr. Jones mentions a paper of Proom and Woiwod<sup>17</sup> in which the authors have come to the "pessimistic" conclusion that in a great number of bacteria examined no new amino acid has been found. We wish to report that, on chromatographing on paper a hydrolyzate of the hydrosoluble portion of a lipo-polysaccharide isolated from *Mycobacterium tuberculosis var. hominis*, we got three spots, two of which are due to glutamic acid and alanine, the third being chromatographically identical with the spot No. 17 which Work<sup>18</sup> has isolated from the proteins of *Corynebacterium diphtheria*. It seems that Dr. Synge has isolated the same substance from the bacteria of the rumen of sheep (private communication). This new compound seems to be a neutral, aliphatic amino acid. We have only found it in the above-mentioned lipo-polysaccharide and not in the proteins of the tubercle bacillus.<sup>19</sup>

There are now available different methods of group separations of amino acids.<sup>20</sup> In a paper with Fromageot and Jutisz<sup>21</sup> we have described in detail such a method, using silica gel for adsorbing the basic amino acids, acid alumina for adsorbing the acidic ones and a special carbon (Activit 50 XP) for the aromatic amino acids. We have checked that this method gives quantitative results also for simple synthetic di- and tripeptides.

On studying recently the behaviour of a synthetic tetrapeptide, kindly put at our disposal by Prof. Prelog (Zurich), we found a somewhat unexpected result. The peptide in question is lysyl-glycyl-glycyl-glutamic acid and thus has a positive charge on one end and a negative charge on the other end of the chain. This substance would probably prove electrophoretically neutral, but it is adsorbed on silica gel as well as on acid alumina. It thus behaves in these group separations as if both charges were distinctly separated.<sup>22</sup>

**Dr. J. A. V. Butler** (London) said: I should like to supplement Dr. Tudor Jones's examples by referring to two uses of chromatography in separating peptides with which I have been concerned. The first was the separation of the smaller peptides produced by the action of chymotrypsin. This was a complex mixture<sup>23</sup> of about 15 peptides of molecular weight of the order of 600. Paper chromatography with phenol and collidine gave, on developing with ninhydrin, a series of coloured bands which merged into each other. When material eluted from different parts of these bands was run again it gave rise to a similar series of coloured bands, so that it seemed that, for example, the portion which ran rapidly at first was distributed between slow- and fast-running fractions in the second run. However, using butanol-acetic acid mixtures two-dimensionally, a separation of about 17 spots with distinctive colours was observed<sup>24</sup> and the separation of this rather complex mixture now looks rather promising with this solvent.

The second example is the concentration of the toxic substance produced by the action of nitrogen trichloride (agene) on flour. It has been shown that the toxic substance is produced by action on the protein constituent of flour. A number of groups have been working<sup>25</sup> on the isolation of the toxic substance

<sup>17</sup> Proom and Woiwod, *J. Gen. Microbiol.*, 1949, **8**, 319.

<sup>18</sup> Work, *Biochim. Biophys. Acta*, 1949, **3**, 400.

<sup>19</sup> Asselineau, Choucrroun and Lederer, *Biochim. Biophys. Acta* (in press).

<sup>20</sup> See the paper of Dr. Jones.

<sup>21</sup> Fromageot, Jutisz and Lederer, *Biochim. Biophys. Acta*, 1948, **2**, 487.

<sup>22</sup> Jutisz (unpublished results).

<sup>23</sup> Butler, Dodds, Phillips and Stephen, *Biochem. J.*, 1948, **42**, 116.

<sup>24</sup> Phillips, *Biochim. Biophys. Acta*, 1949, **3**.

<sup>25</sup> Reiner *et al.*, *Fed. Proc.*, 1949, **8**, 230. Butler and Mills, *Nature*, 1949, **163**, 835. Bentley, Moran *et al.*, *Nature*, 1949, **163**, 675; 1949, **164**, 438.

and their methods, so far as they have been published, involve the digestion of the protein with pancreatin, followed by a chromatographic fractionation of the products. Acid- and base-exchange resins permitted the separation of the fractions rich in acid and basic amino acids, and activated alumina was used to remove some of the neutral amino acids. The selectivity of chromatographic methods is very strikingly illustrated by this work, in which gallons of digest are passed through large columns, in order to obtain finally a very small quantity of the active substance.

**Dr. A. J. Wolwod** (*Beckenham, Kent*) said: The factors responsible for the progressive loss of amino nitrogen as an amino acid passes down the paper must be determined if the colorimetric copper method is to be of use for more than comparative work. If failure of the amino acid to reach equilibrium with the mobile phase is the reason for the loss, then slowing up the rate of running by suitable choice of paper and solvent may effect an improvement. Miettinen<sup>26</sup> reports that very satisfactory separations are obtained by such slow running.

Gordon suggests that a more likely explanation of the loss is reaction with material in the paper. Fowden<sup>27</sup> has found, using the elution technique of Consden, Gordon and Martin,<sup>28</sup> that the longer a paper strip with an amino acid on it is washed the less the apparent recovery as measured by the colorimetric copper method. Using glycine the recovery fell to 51 % after 24-hr. washing. Washings from paper blanks did not affect the reaction between copper and sodium diethyldithiocarbamate and presumably the reaction between copper and amino acid was the one affected. The material responsible may perhaps be a metal forming complexes with amino acids of such stability that it cannot be displaced by copper.

Estimates of the sensitivity of the fluorescent detection technique should be accepted with caution as paper has been found to vary markedly in its ability to permit fluorescence of amino acids. With good papers fluorescence appears to rival ninhydrin in sensitivity in detecting amino acids and peptides.

**Dr. K. W. Pepper** (*Teddington*) said: I should like to amplify the reference made by Dr. Partridge to the use of sulphonated polystyrene resins of different degrees of cross-linking. The results obtained emphasize the importance of polymer structure as a parameter in ion-exchange chromatography and thereby confirm the findings of Kunin and Myers with anion-exchange resins (this Discussion).

Cation-exchange resins were prepared by sulphonating the products obtained from the copolymerization of styrene with different proportions of divinyl benzene. The decrease in swelling resulting from increased cross-linking is shown in the Table.

TABLE I

C.R.L. Reference no.	Nominal DVB Content	Capacity (m.mole/g. dry H <sup>+</sup> resin)	Swelling (g. water/g. dry H <sup>+</sup> resin)	% Volume shrinkage of H <sup>+</sup> form in water to		
				1 N HCl	2 N HCl	5 N HCl
2088	4 %	5.30	1.80	10	15	25
2168	5 %	5.30	1.47	5	12	20
2169	10 %	5.25	0.82	0	4	10

The performance of the above resins in the attempted separation of the more basic amino acids was compared directly, using similar conditions for each run. (Full details of the procedure will appear elsewhere.) After regeneration, the columns were first saturated with 0.1 M glycine hydrochloride in order to minimize changes in ionic strength, which cause shrinkage of the resins and disturbance

<sup>26</sup> Miettinen, *Proc. 1st Int. Congr. Biochem.*, 1949, 229.

<sup>27</sup> Fowden (personal communication).

<sup>28</sup> Consden, Gordon and Martin, *Biochem. J.*, 1947, 40, 590.

of the column. The test solution containing leucine, histidine, lysine and arginine (0.15–0.2 M concentration) was added and subsequently displaced with 0.15 N NaOH (rate of progression of Na<sup>+</sup> boundary, 5 cm./hr.).

The flowing chromatograms obtained by Dr. Partridge are shown diagrammatically in Fig. 1. The influence of polymer structure is strikingly demonstrated

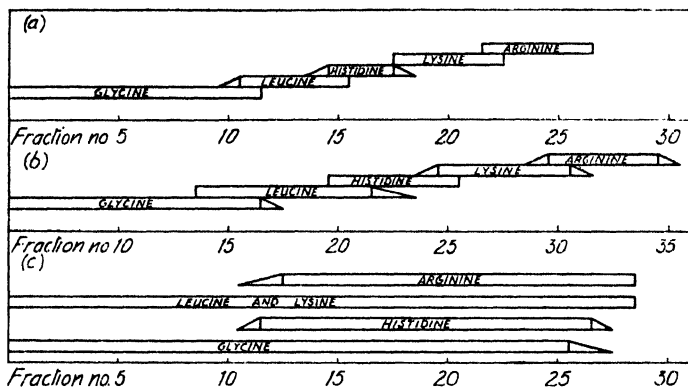


FIG. 1.—Flowing chromatograms obtained by displacing the more basic amino acids from sulphonated polystyrene resins of different degrees of cross-linking.

(a) Sample No. 2088 (4 % DVB).

(b) Sample No. 2168 (5 % DVB).

(c) Sample No. 2169 (10 % DVB).

in that good separations were obtained with the lightly cross-linked resins, whilst the resin containing 10 % DVB was ineffective. A highly cross-linked commercial resin of this type (swelling, 0.75 g. water/g. dry H<sup>+</sup> resin) also proved to be ineffective.

## SUMMARIZING PAPER

By A. J. P. MARTIN

*Received 19th October, 1949*

**General.**—The renaissance of chromatography that has occurred during the last decade has been principally in its extension to water-soluble substances; most of the earlier successful applications being of relatively non-polar substances which were eluted from the adsorbent by such solvents as alcohols. It is noteworthy that in the papers above concerned with the separation of hydrocarbons, carotenoids, steroids and fatty oils, the classical type of adsorbent and solvent is still found to be the most suitable. In the same period, understanding of the ion-exchange properties of variously treated alumina has permitted the separation of many inorganic ions and of various amino acids and their derivatives. Absorbent clays and natural zeolites have also found employment, but the greatest extension has been with synthetic resins deliberately made for their ion-exchange properties. Tiselius and his school have shown how use can be made of the curved adsorption isotherms characteristic of active charcoal in frontal analysis and displacement chromatograms; and so catholic is the adsorption affinity of carbon that, at least in frontal analysis, the method seems applicable

to all classes of soluble substances. Partition chromatography has shown itself capable of separating an extremely wide range of substances, particularly those of biochemical interest. It is perhaps not fanciful to suggest that the success of, say, the butanol-water system for many metabolic products is because they are transported to the cell, and within it, in an aqueous medium, and they have also to pass lipid membranes.

**Specificity.**—The question of specificity of the adsorbent for particular substances or classes of substances has been raised several times, but it is clear that work and ideas on this subject are still in their infancy. So far as metals are concerned a number of more or less specific reagents which form complexes are available, and these can be used directly as in the 8-hydroxyquinoline columns, or as an adjunct in partition or ion-exchange columns. That such molecular groupings should be incorporated in synthetic resins is an attractive idea, though perhaps one not easy to achieve.

An interesting method, not mentioned in the Discussion, is that of Hamoir.<sup>1</sup> Using  $\text{Ag}_2\text{S}$  treated so that the surface was covered with  $\text{Ag}^+$  ions, he found that amino acids which formed insoluble silver salts, or complexes with silver, were preferentially adsorbed; similar results could be shown with  $\text{BaSO}_4$ . The ideal of preparing an adsorbent specific for a particular substance has been attempted by Dickey<sup>2</sup> by precipitation of silica in the presence of the molecule to be preferentially adsorbed, but it is as yet too early to show how widely this can be applied. The high specificity shown by enzymes and antibodies in nature, which indeed inspired the method, is an example of what should ultimately be possible. As a converse of this argument it would seem to be reasonable to attempt to purify enzymes upon columns of the substrate, or some appropriately modified substrate, which should of course for preference be insoluble. The writer has no concrete example except perhaps starch, cellulose or other insoluble polysaccharide, or indeed protein in mind. But no doubt if it could be shown to work in any individual case, other more speculative examples could be tried.

**Crystallization as a Method of Purification.**—It seems relevant at this point to recall that the process of crystallization can be regarded as a special case of adsorption, where the adsorbent remains unaltered, no matter how much solute is adsorbed. In very many cases the specificity is extraordinarily high; classical chemistry has used this method of purification more than any other. No doubt the specificity of the outside unimolecular layer is not as high as within the crystal, since only one side has to fit, and as the crystal grows many of the "wrong" molecules are displaced, since their fit will become progressively worse as they become more nearly enclosed; the slowness of crystallization in complicated mixtures may well be attributed to this necessity for displacement. It is curious that though fractional crystallization has been a long-practised method, no one has published a fractional crystallization method employing a column arrangement. The writer wishes to suggest the following scheme;<sup>3</sup> it cannot perhaps be called a chromatogram though some resemblance is obvious. The apparatus would consist of a long tube packed with some filter aid material, kieselguhr, paper fibres, glass wool, asbestos, etc. Over a fraction of the length of the tube a temperature gradient would be imposed, as by a metal block, hotter at one end than the other. A solvent, in which some at least of the solutes had a high temperature coefficient of solubility, would flow down the tube in the direction hot to cold, and the tube itself be moved through the

<sup>1</sup> Hamoir, *Biochem. J.*, 1945, **39**, 485.

<sup>2</sup> Dickey, *Proc. Nat. Acad. Sci.*, 1949, **35**, 227.

<sup>3</sup> The writer has since heard that Dr. J. Bowman, of the Mellon Institute, has used essentially this method for the separation of waxes.

temperature gradient in the opposite direction. Solid would be placed originally at the hot end of the tube and would be carried to the colder part by movement of the solvent and deposited. The filter aid material would prevent its being carried along the tube as solid. As the tube moved through the temperature gradient the solid would redissolve to reprecipitate again, in approximately the same position relative to the temperature gradient. Continuous recrystallization should thereby be possible with minimal losses in solvent and vessels. Where a number of substances were present, which did not form mixed crystals, only the least soluble would be obtained pure. By a curious paradox where mixed crystals were formed, substantial amounts of each substance should be pure, with regions of overlap as in displacement chromatography. Where temperature coefficients are too small to make this method useful, conceivably gradients of salt concentrations or pH could be devised.

Using paper strips in place of the packed tube, an essentially similar method utilizing evaporation of the solvent suggests itself. A long dry strip of paper would pass through a region where the vapour pressure of the solvent gradually rose to a reservoir of solvent, the flow of solvent in the opposite direction being due to capillarity, with or against gravity. A steady state should eventually be established where substances put on to the paper would collect at characteristic places in the region of the gradient. A similar distillation analogue of the gas-phase chromatogram is obvious.

**Application of the Chromatogram to Large-scale Separations.**—The quantity of material handled on a chromatogram is usually but a small fraction of the mass of the chromatogram itself, and with elution development solutions are normally very dilute. As a result the application of the chromatogram for industrial-scale separation has been limited to special cases, as in water softening where high dilutions are no disadvantage, or where valuable products are concerned. However, with displacement chromatograms relatively high concentrations can be used, and it can be predicted confidently that they will ultimately find wide application. For many industrial processes continuous operation is very desirable. A continuous chromatogram can be envisaged where an adsorbent is forced up a tube counter-current to the solvent with the substances to be separated introduced somewhere between the ends of the tube. The difficulties of obtaining uniform packing and flow of materials are, however, so formidable that no practical arrangement of this type has yet been published. Perhaps if the adsorbent were made in the form of long fibres, e.g., by incorporation in a viscose thread, an endless rope, untwisted or lightly twisted, could be drawn with acceptable uniformity of packing through a column and through appropriate eluting baths to return the rope to its starting condition to re-enter the column.

An idea rising from a discussion with Prof. Tiselius and Dr. Synge during this Discussion can also enable chromatography to be a continuous process, provided that the developing solvent returns the adsorbent to its initial state within a reasonable period. Imagine the chromatogram to be packed within a narrow annular space between two concentric cylinders. The upper surface of the chromatogram is flooded with solvent and at one point the solution to be separated is fed on slowly. The annular chromatogram is slowly and uniformly rotated with the result that different zones will form helices of characteristic angle which can be collected at various fixed points around the bottom of the chromatogram.<sup>4</sup> The annular chromatogram

<sup>4</sup> The writer has since heard that this scheme is already being tried out by Dr. Wadman, of the University of Bristol.

could of course be replaced by a circular array of chromatogram tubes without essential differences, and perhaps less practical difficulty.

**Application of the Chromatogram to Separation of Micro-quantities.**—Scaling down the chromatogram should also be considered. There is perhaps no essentially chromatographic problem here. It is primarily a matter of detection of the substances separated. With radioactive substances this offers little difficulty, and biological methods can detect as little as  $10^{-10}$  g. vitamin B<sub>12</sub> after separation on chromatograms. Nevertheless, using the colour reactions conventional in paper chromatography, replacement of the paper strip by a single yarn of cotton (or perhaps viscose) should result in the reduction of scale by probably a factor of one hundred in the amount of solute required. For ease of observation it would probably be convenient to wrap the yarn round a rod about 1 mm. diam. before or after development of the colour. Another possibility of detection lies in collecting the effluent from the thread in short lengths of thermometer tubing, and determining the amount, after freeze-drying within the tube by absorption of soft X-rays. It should be possible to detect at any rate  $10^{-9}$  g. per sample, and by the use of characteristic wavelengths, following Engstrom,<sup>5</sup> to do an approximate elementary analysis. Observation of the refractive index, so convenient in larger columns, is difficult if the column diameter is reduced to a fraction of a millimetre, but a total reflection method may be applicable since only a thin layer of liquid is required. It should be noted that there is a peculiar gain obtained with very narrow columns, with frontal analysis or displacement development, that lateral diffusion will permit no irregularity of flow to upset the sharpness of front. Hence, provided the observational difficulties could be overcome, micro-columns might well give better analytical results than larger columns.

**Chromatographic Separation of Large Molecules.**—So few studies of chromatographic separations of large molecules have as yet been made that difficulties tend perhaps to be overestimated. Claesson has shown that in spite of slow adsorption and difficulty of elution characteristic of large molecules on carbon, impressive results can be obtained by frontal analysis of high polymers. So far as partition chromatography is concerned, the principal difficulty is likely to be in finding solvent systems in which partition coefficients are not extremely large or small, since as a general rule, for molecules of the same type, the higher the polymer the more it will favour a given phase. Where suitable solvents exist, and kieselguhr can be used as a support for the stationary phase, there is every reason to believe that very large molecules could be successfully separated. It is improbable that cellulose, starch or even precipitated silica would be as effective as kieselguhr as a support, owing to molecular-sieve effects. For these may be expected, not only to reduce adsorption capacity of the column, but also, particularly in the region where the sieve effect is becoming important, to slow down the attainment of equilibrium, and thus reduce the resolving power of the column. On the other hand, with materials such as starch and cellulose, solvents consisting of mixtures of two miscible components may be used and the difference between the stationary and mobile "phases" will more often be small enough to permit of "partition coefficients" of convenient values even for large molecules.

The separation of proteins by chromatography is greatly complicated by the instability of many native proteins. Denaturation seems to occur on most of the common adsorbents though perhaps the use of low tempera-

<sup>5</sup> Engstrom, *Acta Radiol.*, Suppl. LXIII, 1946.

tures to prevent this has not yet been fully explored. There do not appear to be any immiscible phase pairs in which hæmoglobin possesses both stability and a convenient partition coefficient. Even a small percentage of denaturation on passage from one phase to another would quickly, in a chromatogram, result in total destruction, and in a partition chromatogram accumulation of denatured protein at the surface of one phase would probably render this surface impermeable to native protein. The success with which proteins can be crystallized, however, suggests that other more insoluble proteins might be non-denaturing adsorbents.

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